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(54) Title: OLIGOMER CONJUGATES AND THEIR USE

(57) Abstract

This invention contemplates an oligomer conjugate having an oligomer capable of binding to a nucleic acid or protein present in a bacteria, an oligomer uptake enhancer molecule and a spacer attached to the oligomer uptake enhancer molecule and the oligomer. This invention also contemplates enhancing the uptake of an oligomer into a bacterial cell by directly and indirectly attaching an oligomer uptake enhancer molecule to an oligomer to form an oligomer conjugate with enhanced uptake properties. These oligomer conjugates can be used to inhibit bacterial growth *in vivo* and *in vitro*.

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DESCRIPTION

Oligomer Conjugates and Their Use

Cross Reference to Related Application

This is a continuation-in-part application of copending application Serial Number 07/565,307 filed August 9, 1990. The disclosure of this copending application is incorporated by reference into this application.

Technical Field

The present invention relates to Oligomer conjugates and the use of these conjugates to treat prokaryotic infections both in vivo and in vitro.

Background

The use of synthetic oligonucleotides to control gene expression offers the potential for developing highly specific and effective therapies for any disease state known to result from the production of specific gene products. These gene products are responsible for or contribute to, the existence of any disease state or the inhibition of these gene products produces a therapeutic effect. Therapies utilizing synthetic oligonucleotides have a tremendous advantage over traditional drugs because the synthetic oligonucleotides can be designed to alter only the function of a specific gene or class of genes.

Synthetic oligonucleotides have been shown to alter the expression of genes in a variety of biological systems. In the majority of these systems, the synthetic oligonucleotide was designed so that it was complementary to a particular RNA present in the targeted cells. These complementary synthetic oligonucleotides are termed antisense oligonucleotides or antisense therapeutics. When the synthetic oligonucleotide hybridizes to the RNA, the translation of that RNA into protein is inhibited.

The therapeutic potential of these synthetic oligonucleotides depends on their effectiveness in inhibiting the targeted RNA's function, the ability to deliver the oligonucleotide into the cell and the ability to maintain effective concentrations of the synthetic oligonucleotide in the targeted cell for a long enough time to significantly affect the disease state.

One method of increasing the effectiveness of these synthetic oligonucleotides is to increase the half-life of the synthetic oligonucleotide after it has entered the target cell. One method of increasing the half-life of these oligonucleotides is to make them resistant to digestion by nucleases typically found in the target cells.

Methylphosphonates (MP) are oligonucleotide analogs in which the charged phosphate oxygen in each internucleotide linkage is replaced by a methyl group. These agents have a number of unique physical and biochemical properties including (i) the ability to form stable complexes with complementary polynucleotides, (ii) the ability to cross the membrane of eukaryotic cells, and (iii) resistance to hydrolysis by nucleases, making MPs an attractive class of analogs as potential antisense therapeutics. See, for example, Miller, P.S. et al. Biochemistry 18:5134-5143 (1979).

A second method of increasing the effectiveness of antisense therapeutics is to enhance the oligonucleotide's entry into the target cell. The entry enhancement strategies effective in eukaryotic cells do not enhance entry into bacterial cells.

U.S. Patent No. 5,138,045, to Cook and Guinosso, issued August 11, 1992, described oligonucleotides having a polyamine directly attached at the 5' position of the sugar moiety of a nucleoside, preferably a thymidine. The direct attachment of the polyamine functional group to the oligonucleotide is said to confer superior cellular uptake in eukaryotic cells.

Gottkin et al., Bioorg. Khim, 16:514-523 (1990) reported an oligonucleotide having the intercalating agent daunomycin directly attached to the 3' phosphate group of the oligonucleotide and a polymyxim B_1 residue directly attached to the 5' terminal phosphate. The modified oligonucleotide was said to be better sorbed by E. coli cells; however actual uptake (penetration into the cells) of oligonucleotide was not distinguished from membrane binding of oligonucleotide.

Targeting eukaryotic cells with antisense therapeutics represents only a portion of the potential antisense therapeutic applications. Disease states caused by prokaryotic organisms, for example, bacteria, are a significant cause of morbidity and mortality in spite of the many antimicrobial agents currently available. Pathogenic bacteria may be, or may become, resistant to antimicrobial agents of choice and the frequency with which antimicrobial resistance is detected appears to be increasing. See, for example, Neu, H.C. Science 257, 1064-1072 (1992). The basis of successful antimicrobial chemotherapy is the differential toxicity of the therapeutic agent for the microbial target or pathogen, such that the therapeutic agent is much more toxic to the pathogen than it is to cells of the organism that has been infected by the pathogen.

A widely used predictor of in vivo efficacy of an antibacterial agent is whether the agent can inhibit bacterial cell growth in vitro. To date, the efficacy of antisense therapeutics has been limited by the inability to deliver and maintain effective concentrations of the synthetic oligonucleotide within a wild-type pathogenic bacterial cell. In the existing published reports in which antisense Oligomers have been used to inhibit bacterial gene expression in whole cell systems, the condition or type of the bacterial cells used, or the features of the antisense oligonucleotide used indicates that these techniques and oligonucleotides would not be

effective against wild-type pathogenic bacteria in the clinical setting. For example, Jayaraman, K., et al. Proc. Nat'l. Acad. Sci. USA 78:1537-1541 (1981) found methylphosphonate oligonucleotides (MP oligonucleotides) of greater than four nucleotides in length were not accumulated or active against wild type Escherichia coli bacteria, and antisense effects with a MP oligonucleotide of seven nucleotides in length were detectable using a hyper-permeable E. coli mutant, but not using E. coli B cells. Gasparro, F.P., et al., Antisense Research and Development, 1:117-140 (1991), reported that diester oligonucleotides monoadducted with a psoralen moiety had specific antisense effects in E. coli heat shocked to enhance permeability and after irradiation with U.V. light to activate the psoralen. Antisense oligonucleotides without the psoralen adduct demonstrated no effect at similar concentrations.

Using a mutant strain of E. coli that renders it unusually permeable to oligonucleotides, Rahman et al., Antisense Research and Development, 1:319-327 (1991), have reported activity of antisense methylcarbamate oligonucleotides complementary to ribosomal RNA. However, the maximum length of oligonucleotide that effectively entered the mutant, hyper-permeable E. coli cells was only four nucleotides. An oligonucleotide of four nucleotides would have too low an affinity and too low a Tm to be able to form a hybrid with a target nucleic acid that would give practical utility. Also, oligonucleotides less than five nucleotides in length are too short to be used as antisense therapeutics because oligonucleotides of this short length will non-specifically interact with nucleic acids of the organism under treatment at many sites. For example, a complementary sequence to an oligonucleotide of four nucleotides in length will occur in the nucleic acids of an eukaryotic organism every 256 nucleotides, on average. Thus, short oligonucleotides will not exhibit

the selective toxicity required for a successful antisense therapeutic.

Additional studies by Rahman et al., supra, indicated that a short (four nucleotides in length) antisense oligonucleotide when directly conjugated to polyethylene glycol (PEG) increased activity of the antisense oligonucleotide in wild type E. coli. However, as noted above the length of the oligonucleotide used was too short to provide sufficient specificity for use as a therapeutic.

In summary, no report in the literature has shown oligonucleotide uptake or antisense activity against bacteria under in vivo conditions: i.e., use of wild type (non-mutant) bacteria not specifically treated to enhance permeability, and use of a nuclease stable oligonucleotide analog greater than 4 nucleotides in length.

The major theoretical barrier for use of antisense Oligomers as antibacterial agents lies in the ability to achieve and maintain adequate concentrations of active Oligomer inside the bacterial cytoplasm, the site of synthesis, transcription, and translation of the target nucleic acid. To achieve this, the Oligomer must cross the permeability barrier of the bacterial cell envelope. In Gram-positive species, this envelope consists of a thick layer of peptidoglycan external to cytoplasmic membrane, a lipid bilayer structure containing proteins involved in solute transport processes. In Gram-negative bacteria, a thinner layer of peptidoglycan lies external to a cytoplasmic membrane and internal to a second lipid bilayer, the outer membrane. The outer membrane is a highly organized asymmetric bilayer of phospholipid with lipopolysaccharide on the external surface. Also present in the outer membrane are proteins involved in specific and non-specific solute transport processes. The envelopes of some bacteria, notably the Mycoplasma (Mollicutes), do not contain peptidoglycan and consist only of single lipid bilayer cytoplasmic membrane. In

order to cross these permeability barriers, an oligonucleotide therapeutic must either diffuse across these structures or be actively transported by one of the energy dependent mechanisms specific for a variety of substrates. Bacterial membranes do not have specific transport systems for oligonucleotides. Thus, the uptake of an oligonucleotide would have to rely on passive diffusion across the bacterial membrane. In Gram-positive bacteria, the oligonucleotides would not diffuse into the bacterial cell because the relatively hydrophilic nature of oligonucleotides prevents the oligonucleotide from crossing the hydrophobic cytoplasmic membrane. In Gram-negative bacteria, the outer membrane generally excludes oligonucleotides that are longer than three nucleotides. Jayaraman, K. et al., Proc. Nat'l Acad. Sci. USA, 78:1537-1541 (1981).

Phosphodiester antisense oligonucleotides conjugated to poly(L-lysine) have been reported to enhance anti-viral activity in eukaryotic cells. See Lemaitre, M., et al., Nucleosides & Nucleotides 6:311-315 (1987). Other compounds have been directly conjugated to oligonucleotides in attempts to increase the uptake of the oligonucleotide into eukaryotic cells; such compounds include steroids, alkyl groups, lipids, and cholesterol. See respectively, Letsinger, R.L. et al., Proc. Nat'l Acad. Sci USA 86:653-656 (1989), Kabanov, A.V. et al. FEBS Lett. 259:327-330 (1990), Shea, R.G. et al. Nucleic Acid Res. 18:3777-3783 (1990), and Letsinger, R.L. et al., supra.

The aliphatic polyamines spermidine, spermine and putrescine are present in relatively large amounts in prokaryotic cells and are thought to be involved in a number of diverse cellular processes including nucleic acid synthesis and maintenance of structure, protein synthesis, and membrane stability. See Tabor, C.W. and Tabor, H., Microbiological Rev. 49:81-99 (1985). These aliphatic polyamines reportedly are actively transported

by E. coli in a temperature and pH-dependent manner. See Tabor, C.W. and Tabor, H., J. Biol. Chem. 241:3714-3723 (1966). In addition, a number of naturally-occurring antibiotics produced by bacteria and fungi contain covalently linked spermine, spermidine, or a related polyamine. Such molecules include bleomycins, spergualins, tallysomycins, cinodine, galactin, tatumine and edeines. Tabor, C.W. and Tabor, H., Microbiological Rev. 49:81-99 (1985). The role of the polyamine moiety of these molecules has not been determined. Some of these molecules are postulated to act by binding to polyanionic DNA; however the presence of such polyamines in these antibiotics has not been shown to enhance uptake of antibiotic into bacterial cells.

Summary of the Invention

It has now been found that the entry of an Oligomer into bacterial cells may be enhanced by forming an Oligomer conjugate comprising an Oligomer coupled to an Oligomer uptake enhancer molecule. Examples of such molecules include but are not limited to a polyamine or a membrane disruption molecule. These Oligomer conjugates have an enhanced entry rate into bacterial cells giving the Oligomer conjugates the enhanced potency necessary for a useful therapeutic agent.

Oligomer conjugates capable of enhanced entry into bacterial cells are produced by attaching an Oligomer to a polyamine or a membrane disruption molecule. The Oligomer conjugate contains an Oligomer that is from 6 to 50 nucleotides in length. Once inside the bacterial cell, this Oligomer functions as an therapeutic Oligomer and is thus capable of specifically interacting with a bacterial nucleic acid sequence or protein present in the target bacterial cell. These therapeutic Oligomers can be designed to be complementary to a wide variety of nucleic acids found in the target bacterial cell. The therapeutic Oligomer can also be designed to specifically interact

with a protein found in the target bacteria. An antisense Oligomer directed against specific prokaryotic nucleic acids provides a specific and highly flexible method to control microbial diseases.

Suitable Oligomer uptake enhancer molecules include but are not limited to molecules which facilitate uptake of the Oligomer conjugate and include polyamines, membrane disruption molecules, and other molecules which facilitate transport across cell membranes and/or cell walls.

The Oligomer conjugate may contain as an Oligomer uptake enhancer molecule a polyamine such as spermidine or spermine. In some embodiments, these polyamines are coupled to an Oligomer via a spacer that is from 1 to 30 atoms in length and have a significantly increased rate of Oligomer entry into bacteria cells as compared to the Oligomer alone.

The Oligomer conjugate may contain as an Oligomer uptake enhancer molecule a membrane disruption molecule such as a polymyxin, octapeptin, pore-forming cyclic peptides, membrane pore-forming linear peptides, other membrane disrupting peptides, ionophores, polymyxin-derived peptides, polylysines, polyornithines, protamine, aminoglycosides, compound 48/80, Tris, EDTA, polyenes and the like.

In the Oligomer conjugates of the present invention, the Oligomer and Oligomer uptake enhancer molecule present in the Oligomer conjugate are linked together via a spacer such as a spacer having from 1 to 30 atoms. The spacer places the Oligomer and the Oligomer uptake enhancer molecule farther apart than they would be if the Oligomer and the Oligomer uptake enhancer molecule were directly linked together. The combination of Oligomer and Oligomer uptake enhancer molecule unexpectedly increases the Oligomer conjugate's ability to enter cells, especially bacterial cells.

According to an alternate aspect, the present invention is directed to methods of increasing Oligomer

uptake in bacteria using an Oligomer directly linked to an Oligomer uptake enhancer molecule.

An increased entry rate into cells makes the Oligomer conjugates useful for therapeutic applications. For example, additional selective toxicity may be designed into the Oligomer conjugate by selecting an Oligomer that is complementary to a nucleic acid sequence present in the target bacterial cells but not present in the eukaryotic cells that make up the organism being treated. According to an especially preferred aspect, an Oligomer is selected that is complementary to a sequence present in the target bacterial cells which is not present in beneficial bacterial cells (such as intestinal flora normally present in mammals). The Oligomer conjugate of the present invention may be used to inhibit the growth of bacteria or to kill bacteria present in a organism such as an animal. The Oligomer conjugates of the present invention may also be used prophylactically to prevent bacterial infection of an organism by administering the Oligomer conjugate to the animal prior to and/or during exposure to a bacterial organism. The Oligomer conjugates may also be used to inhibit bacterial growth <u>in vitro</u> in tissue culture, fermentation processes, the environment or on the surface of an implantable medical device.

According to one aspect, the present invention provides compositions containing a pharmaceutically acceptable and therapeutically effective amount of an Oligomer conjugate that is capable of hybridizing to a nucleic acid of a bacteria. The compositions are useful in the treatment or prevention of bacterial infections and to kill bacteria present in an animal, on a surface, or in the environment.

According to one aspect, the present invention provides methods of enhancing uptake of antisense Oligomers by target cells, particularly prokaryotic cells which heretofore have shown only poor uptake due to

inability of such antisense Oligomers to get through the cell wall.

The Oligomer conjugates of the present invention when used to treat an animal are given in a sufficient concentration to establish a therapeutic concentration within the animal. These therapeutic Oligomer conjugates typically contain Oligomers that are complementary to part of an RNA transcript or DNA segment that is present in the bacterial pathogen and thus capable of inhibiting the growth of the pathogen. This therapeutic concentration is maintained within the animal for a long enough time period to treat the animal by inhibiting the growth of the target bacterial pathogen.

According to a further aspect, the present invention also provides a method of introducing an Oligomer conjugate into a bacterial cell by contacting the Oligomer conjugate with a bacterial cell under conditions that support its accumulation inside the bacterial cell. The contact between the Oligomer conjugate and the cell is maintained for a time period that is sufficient for transfer of the Oligomer conjugate into the cell.

The present invention also provides therapeutic agents containing Oligomer conjugates that are useful for inhibiting the growth of bacteria by altering the function of a nucleic acid or protein within the bacterial cell. The Oligomers that are part of these therapeutic agents bind to either a nucleic acid or protein present in the bacterial cell and alter its function thereby inhibiting bacterial growth.

Brief Description of the Drawings

In the drawings forming part of this disclosure:

Figure 1 depicts a chemical formula of an Oligomer conjugate is shown, including the Oligomer, the spacer and the polyamine or membrane disruption molecule.

Figure 2 depicts an example of synthesis of an Oligomer conjugate. Figure 2A is a diagram of the

starting Oligomer having a portion of the spacer previously inserted between nucleosides and the DSS that will form the remainder of the spacer. Figure 2B is a diagram of the reaction of the Oligomer having the spacer with the polyamine. Figure 2C is a diagram of the final Oligomer conjugate formed by the above reaction.

Figure 3A and 3B depict cleaving groups useful in conjugating a spacer to a polyamine or membrane disruption molecule.

Detailed Description of the Invention

A. Definitions

DNA: Deoxyribonucleic acid.

RNA: Ribonucleic acid.

rRNA: Ribosomal RNA.

Hydrocarbyl: An organic radical composed of carbon and hydrogen which may be aliphatic (including alkyl, alkenyl, and alkynyl groups and groups which have a mixture of saturated and unsaturated bonds), alicyclic (carbocyclic), aryl (aromatic) or combinations thereof; and may refer to straight-chained, branched-chain, or cyclic structures or to radicals having a combination thereof, as well as to radicals substituted with halogen atom(s) or heteroatoms, such as nitrogen, oxygen, and sulfur and their functional groups (such as amino, alkoxy, aryloxy, lactone groups, and the like), which are commonly found in organic compounds and radicals. Nucleotide: A subunit of a nucleic acid comprising a phosphate group, a 5-carbon sugar and a nitrogen containing base. In RNA, the 5-carbon sugar is ribose. In DNA, it is a 2-deoxyribose. The term also includes analogs of such subunits.

Nucleoside: Includes a nucleosidyl unit and is used interchangeably therewith, and refers to a subunit of a nucleic acid which comprises a 5-carbon sugar and a nitrogen-containing base. The term includes not only those nucleosidyl units having A, G, C, T and U as their bases, but also analogs and modified forms of the

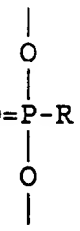
naturally-occurring bases, such as pseudoisocytosine and pseudouracil and other modified bases (such as 8-substituted purines). In RNA, the 5-carbon sugar is ribose; in DNA, it is 2'-deoxyribose. The term nucleoside also includes other analogs of such subunits, including those which have modified sugars such as 2'-O- alkyl ribose.

Nucleotide multimer: A chain of nucleotides linked by phosphodiester internucleosidyl linkages or other internucleosidyl linkages. Such internucleosidyl linkages include not only phosphorus-containing linkages such as alkyl and/or aryl phosphonate linkages, alkyl and/or aryl phosphonothioates, phosphorothioate or phosphorodithioates, neutral phosphate ester linkages such as phosphotriesters, phosphoramidite linkages and other reported analogs. One or more of the phosphorus-containing linkages may be replaced by a non-phosphorus linkage such as a formacetal linkage, a thioformacetal linkage, a sulfamate linkage or a carbamate linkage.

Nucleoside base unit: A unit of a nucleotide multimer or Oligomer that contains one nucleoside. Thus, nucleotide multimers and Oligomers have a length measured in nucleoside base units that is equivalent to the number of nucleosides that make up the multimer or oligomer.

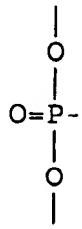
Polynucleotide: A nucleotide multimer generally about 100 nucleotides or more in length. These are usually of biological origin or are obtained by enzymatic means.

Phosphonate: The group



wherein R is hydrogen or an alkyl or aryl group. Suitable alkyl or aryl groups include those which do not sterically hinder the phosphonate linkage or interact with each other. The phosphonate group may exist in either an "R" or an "S" configuration. Phosphonate groups may be used

as internucleosidyl phosphorus group linkages (or links) to connect nucleosidyl units.



Phosphodiester: The group

wherein phosphodiester groups may be used as internucleosidyl phosphorus group linkages (or links) to connect nucleosidyl units.

Non-nucleoside monomeric unit: A monomeric unit wherein the base, the sugar and/or the phosphorus backbone or other internucleosidyl linkage group of a nucleoside has been replaced by other chemical moieties.

Nucleoside/non-nucleoside polymer: A polymer comprised of nucleoside and non-nucleoside monomeric units.

Oligonucleoside or Oligomer: A chain of nucleosides which are linked by internucleoside linkages which is generally from about 4 to about 100 nucleosides in length, but which may be greater than about 100 nucleosides in length. They are usually synthesized from nucleoside monomers, but may also be obtained by enzymatic means. Thus, the term "Oligomer" refers to a chain of nucleosides which have internucleosidyl linkages linking the nucleoside monomers and, thus, includes oligonucleotides, nonionic oligonucleoside alkyl- and aryl-phosphonate analogs, alkyl- and aryl-phosphonothioates, phosphorothioate or phosphorodithioate analogs of oligonucleotides, phosphoramidate analogs of oligonucleotides, neutral phosphate ester oligonucleoside analogs, such as phosphotriesters and other oligonucleoside analogs and modified oligonucleosides, and also includes nucleoside/non-nucleoside polymers. The term also includes nucleoside/non-nucleoside polymers wherein one or more of the phosphorus group linkages between monomeric units has been replaced by a non-phosphorous linkage such as a formacetal linkage, a thioformacetal linkage, a

sulfamate linkage, or a carbamate linkage. It also includes nucleoside/non-nucleoside polymers wherein both the sugar and the phosphorous moiety have been replaced or modified such as morpholino base analogs, or polyamide base analogs. It also includes nucleoside/non-nucleoside polymers wherein the base, the sugar, and the phosphate backbone of a nucleoside are either replaced by a non-nucleoside moiety or wherein a non-nucleoside moiety is inserted into the nucleoside/non-nucleoside polymer. Optionally, said non-nucleoside moiety may serve to link other small molecules which may interact with target sequences or alter uptake into target cells.

Alkyl or Aryl-phosphonate Oligomer: An Oligomer having at least one alkyl- or aryl-phosphonate internucleosidyl linkage. Suitable alkyl- or aryl- phosphonate groups include alkyl- or aryl- groups which do not sterically hinder the phosphonate linkage or interact with each other. Preferred alkyl groups include lower alkyl groups having from about 1 to about 6 carbon atoms. Suitable aryl groups have at least one ring having a conjugated pi electron system and include carbocyclic aryl and heterocyclic aryl groups, which may be optionally substituted and preferably having up to about 10 carbon atoms.

Methylphosphonate Oligomer (or "MP-Oligomer"): An Oligomer having at least one methylphosphonate internucleosidyl linkage.

Neutral Oligomer: An Oligomer which has nonionic internucleosidyl linkages between nucleoside monomers (i.e., linkages having no positive or negative ionic charge) and includes, for example, Oligomers having internucleosidyl linkages such as alkyl- or aryl-phosphonate linkages, alkyl- or aryl-phosphonothioates, neutral phosphate ester linkages such as phosphotriester linkages, especially neutral ethyltriester linkages; and non-phosphorus-containing internucleosidyl linkages, such as sulfamate, morpholino, formacetal, thioformacetal, and

carbamate linkages. Optionally, a neutral Oligomer may comprise a conjugate between an oligonucleoside or nucleoside/non-nucleoside polymer and a second molecule which comprises a conjugation partner. Such conjugation partners may comprise intercalators, alkylating agents, binding substances for cell surface receptors, lipophilic agents, nucleic acid modifying groups including photo-crosslinking agents such as psoralen and groups capable of cleaving a targeted portion of a nucleic acid, and the like. Such conjugation partners may further enhance the uptake of the Oligomer, modify the interaction of the Oligomer with the target sequence, or alter the pharmacokinetic distribution of the Oligomer. The essential requirement is that the oligonucleoside or nucleoside/non-nucleoside polymer that the Oligomer conjugate includes be substantially neutral and be capable of hybridizing to its complementary target sequence.

Substantially neutral: An Oligomer refers to those Oligomers in which at least about 80 percent of the internucleosidyl linkages between the nucleoside monomers are nonionic linkages.

Neutral alkyl- or aryl- phosphonate Oligomer: Neutral Oligomers having neutral internucleosidyl linkages which comprise at least one alkyl- or aryl- phosphonate linkage.

Neutral methylphosphonate Oligomer: Neutral Oligomers having internucleosidyl linkages which comprise at least one methylphosphonate linkage.

Polypeptide and Peptide: A linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

Protein: A linear series of greater than about 50 amino acid residues connected one to the other as in a polypeptide.

Gene: A segment of DNA coding for an RNA transcript that is itself a structural RNA, such as ribosomal RNA or codes for a polypeptide. The segment of DNA is also equipped

with a suitable promoter, termination sequence and optionally other regulatory DNA sequences.

Structural Gene: A gene coding for a structural RNA and being equipped with a suitable promoter, termination sequence and optionally other regulatory DNA sequences.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Therapeutic Oligomer: An Oligomer that is present alone or as part of an Oligomer conjugate and is capable of binding to a protein of a target bacterial cell or to a nucleic acid present in the target bacterial cell and thereby affecting the growth of the target bacterial cell. A therapeutic Oligomer that binds to a nucleic acid present in the target bacterial cell is an antisense therapeutic.

Oligomer Uptake Enhancer Molecule: An Oligomer uptake enhancer molecule is a molecule which, when convalently attached to an Oligomer, either via a spacer or directly, as part of an Oligomer conjugate, facilitates uptake of the Oligomer conjugate by the target cells as compared to the unconjugated Oligomer. Accordingly, Oligomer uptake enhancer molecules may be selected to exploit any of the mechanisms by which molecules are internalized by cells, especially bacterial cells, such as passive diffusion, facilitated diffusion, active transport, group translocation, membrane disruption or the so-called "self-promoted" uptake pathway or a combination of such mechanisms. See, e.g., Nikaido, H., et al., Science 258:936-942 (1992) and Hancock, R.E.W., et al., Eur. J. Clin. Microbiol. Infect. Dis. 7:713-720 (1988).

Membrane Disruption Molecule: Membrane disruption mole-

cules physically disrupt biological membranes, such as bacterial cell membranes and eukaryotic cell membranes, by forming a pore or channel in the biological membrane or by altering the structure of the biological membrane. Membrane disruption molecules include the membrane disruption molecules described in Vaara, M. et al., Microbiol. Revs. 56(3):935-411 (1992) and Gale, E.F. et al., The Molecular Basis of Antibiotic Action, John Wiley and Sons, London, 1972.

Blocking Group: A blocking group is a group which is attached to either the 5'-oxygen or 3'-oxygen of a nucleoside or the equivalent position of a non-nucleotide monomeric unit which prevents reaction of the oxygen (during certain steps of Oligomer synthesis) but which is removable under non-adverse deblocking conditions.

Deblocking Conditions: Deblocking conditions describe the conditions used to remove the blocking (or protecting) group.

Non-Adverse Conditions: Non-adverse conditions describes conditions (of reaction or synthesis) which do not substantially adversely affect the Oligomer skeleton and its sugar, and base components, nor the solid support. One skilled in the art can readily identify functionalities, coupling methods, deblocking and deprotection procedures and cleavage conditions which meet these criteria.

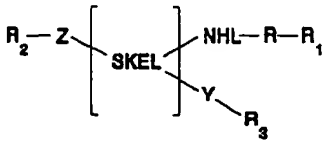
B. Oligomer Conjugates

The Oligomer conjugates of the present invention contain at least an Oligomer, a spacer and an Oligomer uptake enhancer molecule and have an enhanced rate of Oligomer conjugate uptake into a bacterial cell. This enhanced uptake can be ascertained by comparing the rate of unconjugated Oligomer (either Oligomer alone or Oligomer-spacer) uptake into a bacterial cell to the rate of uptake of an Oligomer conjugate into a bacterial cell.

The present invention also provides novel Oligomer conjugates containing an Oligomer, an Oligomer uptake enhancer molecule and a spacer attached to the Oligomer and to the Oligomer uptake enhancer molecule. Typically, each Oligomer conjugate contains one Oligomer uptake enhancer molecule attached to one Oligomer via a single spacer. However, the present invention also contemplates Oligomer conjugates containing more than one spacer so that the Oligomer uptake enhancer molecule is attached to the Oligomer at more than one position. Other Oligomer conjugates containing more than one spacer, at least two Oligomers and more than one Oligomer uptake enhancer molecule are also contemplated.

Oligomer conjugates containing more than one Oligomer may have two of the same Oligomers or two Oligomers that are different in at least one respect such as in nucleoside base sequence of the Oligomers. These Oligomers would be different if, for example, they were composed of different nucleotide sequences, contained different types of linkages between nucleotides or contained various nucleotide analogs. If more than one spacer is present, the spacers may be the same, similar or different. If more than one molecule is present in an Oligomer conjugate, the Oligomer uptake enhancer molecules may be the same, similar, or different.

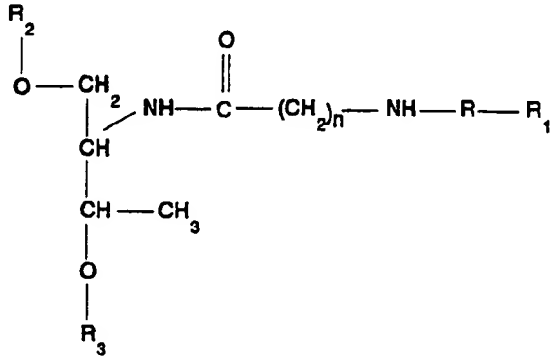
According to a preferred aspect, the present invention provides an Oligomer conjugate having from about 6 to 50 nucleosides of the formula I:



wherein SKEL comprises a skeleton of about 1 to about 20 carbon atoms, wherein NHL, Y and Z are covalently linked to a carbon atom of SKEL; L is hydrocarbyl or a bond; Y is

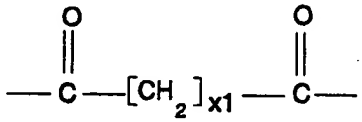
$-CH_2-$, -O-, -S-, or -NH-; and Z is -O-, -S-, or -NH-; R is hydrocarbyl or a bond; R_1 is an Oligomer uptake enhancer molecule; R_2 and R_3 are independently selected from the group consisting of a nucleoside, a nucleoside of an Oligomer, hydrogen and a blocking group, provided that R_2 and R_3 taken together comprise at least 6 nucleosides. According an especially preferred aspect, SKEL is chirally pure.

According to an especially preferred aspect, the present invention provides an Oligomer conjugate of the formula II:



II

wherein n is an integer from 1 to 6; R is hydrocarbyl of 1 to 20 carbon atoms; R_1 is an Oligomer uptake enhancer molecule; and R_2 and R_3 are independently selected from the group consisting of a nucleoside, a nucleoside of an Oligomer and a blocking group. More preferably R is



wherein X_1 is an integer from 1 to 12.

The general structure of an especially preferred Oligomer conjugate containing an Oligomer uptake enhancer molecule which is a polyamine is shown in Figure 1. The Oligomer is typically attached to the spacer via the 3' or 5' position of a nucleotide of the Oligomer. Such an attachment may insert the spacer between two nucleosides

of an Oligomer. The present invention also contemplates spacers attached to only the 5' most nucleoside or the 3' most nucleoside of an Oligomer. However, linkages at other positions of a nucleoside of an Oligomer are well known and would be useful in the present invention. These alternative linkages between the Oligomer and the spacer would be useful, provided that the linkage allows the Oligomer conjugate to fulfill its function. Optionally other Oligomer uptake enhancer molecule(s) may replace one or more of the pictured polyamines present in the conjugate.

The spacer is attached to the both Oligomer and to the Oligomer uptake enhancer molecule. The composition and length of the spacer may vary so long as the resulting Oligomer conjugate has its desired function. The function of the Oligomer conjugate can be readily ascertained by determining its uptake into bacterial cell according to the methods described in the example section of this application. The linkage between the spacer and the Oligomer uptake enhancer molecule is typically between a nitrogen atom of the Oligomer uptake enhancer molecule and a carbon atom of the spacer. One skilled in the chemical arts will understand that various types of linkages between the spacer and the Oligomer uptake enhancer molecule are possible and thus contemplated by this invention.

Oligomer conjugates having repeated units of the entire structure shown in Figure 1 (Oligomer-spacer-Oligomer uptake enhancer molecule) are also provided by the present invention. Oligomer conjugates in which more than one spacer-Oligomer uptake enhancer portion of the structure is attached to an Oligomer are also provided. Oligomer conjugates having more than one spacer attaching the Oligomer to an Oligomer uptake enhancer molecule are also contemplated by this invention.

Spacer molecules and portions of the spacer molecules suitable for the Oligomer conjugates of the present

invention and their syntheses are well known and such spacers include the non-nucleotide reagents disclosed by Reynolds et al., PCT Publication No. WO 92/02532 (1992), Arnold et al., PCT Publication No. WO 89/02933 and Arnold et al., PCT Publication No. 89/02439 (1989) the disclosures of which are incorporated herein by reference. These spacers, or portions of spacers, may be coupled into an Oligomer using nucleic acid synthesis techniques that are known in the art. The spacers, when coupled to an Oligomer, contain nucleophilic primary amines through which a variety of secondary compounds may be attached by standard aqueous chemistries in the art. Examples of useful chemistries suitable for linking secondary compounds to the portion of the spacer incorporated into the Oligomer include the reaction of alkylamines with active esters, active imines, arylhalides, or isothiocyanates, and the reaction of thiols with maleimides, haloacetyls, etc. (For further potential techniques see G.M. Means and R.E. Feeney, "Chemical Modification of Proteins", Holden-Day Inc., 1971; R.E. Feeney, Int. J. Peptide Protein Res., Vol. 29, 1987, p. 145-161). Suitable protecting groups or blocking groups which can be used to protect the functional group on the portion of the spacer during incorporation into an Oligomer are also similar to those used in protein chemistry (see for example, "The Peptides: Analysis and Synthesis, Biology," Vol. 3, ed. E. Gross and J. Meienhofer, Academic Press, 1971).

Particularly useful for the synthesis of spacers are secondary compounds with reactive groups that can extend the spacer and also be used to couple the Oligomer uptake enhancer molecules. A number of preferred coupling moieties for -OH, -SH, and $-NH_2$ are listed in Figures 3A and 3B. Secondary molecules may contain one or two leaving groups, and if two leaving groups are present, the polyamine may be attached in a single step. If the secondary molecule contains only one leaving group and

that group is utilized to attach to the portion of the spacer molecule incorporated into the Oligomer, an activation step is required before the Oligomer uptake enhancer molecule may be attached. The secondary molecule once attached to the portion of the spacer incorporated into the Oligomer becomes part of the spacer that connects the Oligomer to the Oligomer uptake enhancer molecule.

Multiple Oligomers may be present in an Oligomer conjugate. This may be achieved by attaching multiple Oligomers to each other or to a spacer or to the Oligomer uptake enhancer molecule.

The Oligomer conjugates of the present invention typically have a ratio of Oligomer to spacer to Oligomer uptake enhancer of 1 Oligomer to 1 spacer to 1 Oligomer uptake enhancer. However, in other embodiments of the invention, this ratio may vary and includes Oligomer conjugates in which this ratio is, for example, 2 to 2 to 1, 1 to 2 to 2, 1 to 3 to 3, 1 to 4 to 4. Useful Oligomer conjugates will have ratios of about 1 to 10 Oligomers, to about 1 to 10 spacers, to about 1 to 10 Oligomer uptake enhancer. Similar variation in the ratios of Oligomer to spacer to Oligomer uptake enhancer molecules are contemplated.

In other embodiments, the Oligomer conjugate has a ratio of Oligomer to Oligomer uptake enhancer molecule that is from 1 to 1 up to and including Oligomer conjugates in which this ratio is 1 to 10. This includes Oligomer conjugates that have, for example, Oligomer to Oligomer uptake enhancer molecule ratios of 2 to 2, 3 to 3, 4 to 4, 5 to 5, 6 to 6, 1 to 3, 1 to 2, 1 to 5, 3 to 1, 4 to 1, etc.

In other embodiments, the Oligomer conjugate used according to the methods of the present invention has a ratio of Oligomer to Oligomer uptake enhancer molecule that is from 1 to 1 up to 1 to 10.

1. Oligomers

The Oligomer conjugates of the present invention contain Oligomers having a length from about 5 to about 50 nucleotide base units. These Oligomers are of sufficient length to be useful in hybridizing to a nucleic acid or binding to a protein within a bacterial cell and affecting the function of the nucleic acid or protein present in the bacterial cell. In some preferred embodiments, the Oligomers have a length from about 6 to about 100 nucleoside units. In preferred embodiments the Oligomers have a length from about 6 to about 50 nucleosides units. In more preferred embodiments of the present invention the Oligomers have a length from about 8 to about 30 nucleoside units. In the most preferred embodiments the Oligomers have a length from about 10 to about 25 nucleoside units. One skilled in the art will understand that the length of the Oligomer present can be selected so that the function of the Oligomer inside a bacterial cell can be optimized. The parameters affecting the function of an Oligomer and its ability to bind a nucleic acid or protein within a bacterial cell are well known in the art and include for example, the base composition of a particular Oligomer, its length and the amount of the target nucleic acid or protein found in the bacterial cell.

The Oligomer conjugates of the present invention may contain structural modifications that provide enhanced utility in biological systems. For example, Oligomers containing altered linkages may be more resistant to nucleases present in serum, mammalian cells and bacterial cells. This nuclease resistance allows the Oligomers to remain active for longer periods of time. Examples of useful Oligomers are the methylphoshonates described by C.H. Marcus-Sekura et al., Nuc. Acids Res. 15:5749-5763 (1987) and P.S. Miller et al., Biochemistry 20:1874-1880 (1981); the phophorothioates described by M. Ceruzzi and K. Draper, Nucleotides and Nucleotides 8:815-818 (1989)

and P.S. Miller et al., Biochem. 16:1988-1996 (1977). The references cited herein are incorporated by reference.

Other useful Oligomers are substantially neutral. Substantially neutral Oligomers have at least 80% of the internucleosidyl linkages present in the Oligomer neutral or non-ionic. In preferred embodiments, the normal phosphodiester backbone of the Oligomer is replaced by a non-ionic methylphosphonate backbone. The use of oligo-2'-deoxyribonucleoside methylphosphonates as antisense inhibitors of mRNA translation in processing has been described in the literature. For example, see Miller et al., Nucleosides and Nucleotides 10:37-46 (1991).

An Oligomer conjugate of this invention typically contains an Oligomer that is complementary to a nucleic acid or capable of binding a protein that is found in a bacterial cell. According to a preferred embodiment, the Oligomer is capable of binding to a nucleic acid or protein found in a bacteria that infects or colonizes an organism such as an animal. In other preferred embodiments, the Oligomer binds to a nucleic acid or protein found in a Gram-negative bacteria. Gram-negative bacteria include Neisseria species such as Neisseria meningitidis, Neisseria gonorrhoeae, Neisseria catarrhalis, Neisseria sicca, and Neisseria flavescens; Salmonella species including Salmonella paratyphi-A, Salmonella schottmulleri, Salmonella typhimurium, Salmonella paratyphi-C, Salmonella cholerae-suis, Salmonella montevideo, Salmonella newport, Salmonella typhi, Salmonella enteritidis, Salmonella gallinarum, Salmonella anatum, Salmonella newington, and Salmonella minneapolis; Escherichia species, including Escherichia coli; Klebsiella species, including Klebsiella pneumoniae; Enterobacter species including Enterobacter cloacae, Enterobacter aerogenes, Enterobacter hafniae, and Enterobacter agglomerans; Serratia species including Serratia marcescens, Serratia liquefaciens, and Serratia rubidaea; Shigellae species including Shigella dysenteriae, Shigella

flexneri, Shigella boydii, and Shigella sonnei; Lepto-trichia species; Bacteroides species including Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides bulgatus, Bacteroides thetaiotaomicron, Bacteroides distasonis, Bacteroides ovatus, Bacteroides oralis, Bacteroides corrodens, and Bacteroides melaninogenicus including subspecies melaninogenicus, subspecies inter-medius, subspecies asaccharolyticus; Fusobacterium species including Fusobacterium nucleatum, Fusobacterium necrophorum, Fusobacterium parium, and Fusobacterium mortiferum; Proteus species including Proteus vulgaris, Proteus mirabilis, Morganella morganii (Proteus morganii), and Proteus rettgeri; Providencia species including Providencia alcalifaciens, Providencia stuartii; Arizona species including Arizona hinshawii; Edwardsiella species including Edwardsiella tarda; Citrobacter species including Citrobacter freundii and Citrobacter diversus; all members of the Enterobacteriaceae; all members of Vibrionaceae including Vibrio species, Vibrio cholerae, Vibrio comma, Vibrio parahaemolyticus; Aeromonas species including Aeromonas hydrophila, and Aeromonas (Ples-iomonas) shigelloides; Campylobacter species including Campylobacter fetus, Campylobacter fetus var. intestinalis, and Campylobacter fetus var. jejuni; Pseudomonas species including Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas pseudomallei, Pseudomonas mallei, Pseudomonas cepacia, Pseudomonas maltophilia, Pseudomonas stutzeri, and Pseudomonas acidovorans; Acinetobacter species including Acinetobacter anintratum (Herellea), Acinetobacter iwoffi (Mina); Flavobacterium species including Flavobacterium meningosepticum; Alcaligenes species including Alcaligenes faecalis; Achromobacter species including Achromobacter xylosoxidans; Moraxella species including Moraxella lacunata, Moraxella osloensis, Moraxella nonliquefaciens (Mima polymorpha var. oxidans), Moraxella catarrhalis and Moraxella phenylpyruvica; Yersinia species including

Yersinia pestis, Yersinia pseudotuberculosis, and Yersinia enterocolitica; Francisella species including Francisella tularensis; Pasteurella species including Pasteurella multocida, Pasteurella pneumogropica, and Pasteurella ureae; Brucella species including Brucella melitensis, Brucella abortus, Brucella suis, Brucella ovis, Brucella neotomae, and Brucella canis; Actinobacillus species including Actinobacillus actinomycetem-comitans; Hemophilus species including Hemophilus influenzae, Hemophilus aegyptius, Hemophilus suis, Hemophilus haemolyticus, Hemophilus ducreyi, Hemophilus aphrophilus, Hemophilus parainfluenzae, and Hemophilus parahaemolyticus; Bordetella species including Bordetella pertussis, Bordetella parapertussis, and Bordetella bronchiseptica; Legionella species including, Legionella pneumophila; Streptobacillus species including Streptobacillus moniliformis; Calymmatobacterium (Donovania) granulomatis; Bartonella bacilliformis; Veillonella species; Eikenella corrodens; Capnocytophaga species; Selenomonas sputigena; and Helicobacter species, including Helicobacter pylori.

According to other preferred embodiments, the Oligomer binds to a nucleic acid or protein found in a gram-positive bacteria. Gram positive bacteria include: Corynebacterium species, including Corynebacterium diphtheriae, Corynebacterium xerosis and Corynebacterium hofnanni; Streptococcus species including Streptococcus pneumoniae, Streptoccus pyogenes, Streptococcus salivarious, Streptoccus faecalislen and β hemolytic Streptococcus; Staphylococcus species including, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, and Staphylococcus saprophyticus; Bacillus species including Bacillus anthracis, Bacillus cereus, Bacillus subtilis, Bacillus brevis, Bacillus neyciterium, and Bacillus polymyxa; Clostridium species including Clostridium botulinum, Clostridium botulus, Clostridium perfringens, Clostridium nouyi, Clostridium difficile, Clostridium tetani,

Clostridium septicum, Clostridium histolyticum, Clostridium tertium, Clostridium bifermentans and Clostridium sporogenes; Mycobacterium species including Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium simiae, Mycobacterium marinum, Mycobacterium scrofulacem, Mycobacterium szulgai, Mycobacterium gordonae, Mycobacterium flavencens, Mycobacterium avium-intracellulare, Mycobacterium xenopi, Mycobacterium gastri, Mycobacterium terrae-triviale, Mycobacterium fortuitum, Mycobacterium chelonei, Mycobacterium leprae, Mycobacterium ulcerans, Mycobacterium lepraemurium, Mycobacterium fortuitum-chelonei; and Mycobacterium smegmatis; Enterococcus species including Enterococcus faecium and Enterococcus faecalis. In other preferred embodiments, the Oligomer binds to a nucleic acid or protein found in a rickettsia, mollicute, yeast, fungus or virus.

One skilled in the art will understand that for these Oligomer conjugates to act as therapeutic Oligomers, the Oligomers present must be complementary to a nucleic acid or interact with a protein present in the bacteria that is important for bacterial growth. When a bacterial nucleic acid or protein that is important for bacterial growth is inhibited, for example by the binding of an Oligomer conjugate to it, bacterial growth is affected. Using selected Oligomers, one skilled in the art will be able to inhibit bacterial growth and in some embodiments inhibit the growth of the bacteria to a sufficient degree so as to kill the bacterial cell.

In preferred embodiments, the Oligomer present in the Oligomer conjugate is complementary to an RNA molecule in the bacterial cell (target RNA sequence). Examples of suitable target RNA sequences include RNA molecules transcribed from a portion of the bacterial chromosome such as messenger RNA, ribosomal RNA, and tRNA molecules, and also RNA molecules transcribed from a portion of an

extrachromosomal DNA element. Extrachromosomal DNA elements include plasmid DNA and viral DNA that is not integrated in the bacterial chromosome. Transposons and viruses may have their DNA integrated into the bacterial chromosome during some portion of their life cycle and during this period they are part of the bacterial genome. During other portions of their life cycle, the transposons and viruses may exist as extrachromosomal DNA elements within the bacterial cell.

In other preferred embodiments, the Oligomer present in the Oligomer conjugate is capable of interacting with a protein that is present inside a bacterial cell and, thus, may alter the function of that protein. Examples of proteins that interact with Oligomers or nucleic acid segments include regulatory proteins, ribosomal proteins, enzymes, nucleic acid synthesis enzymes, enzymes that act on DNA or RNA and the transfer RNA synthetases.

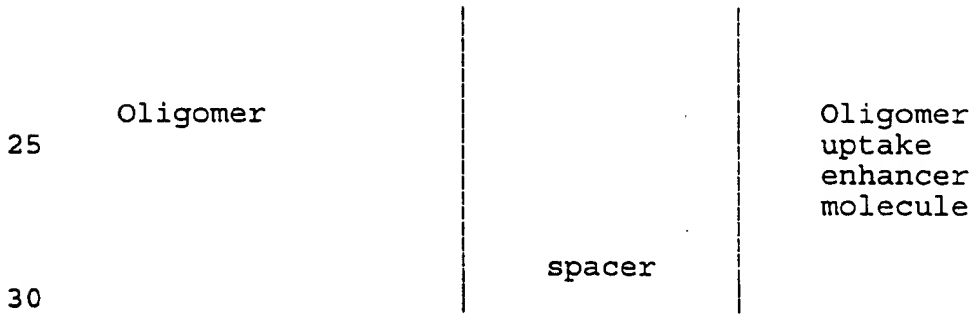
One skilled in the art will understand that many portions of an RNA present in a cell may be selected as an RNA target sequence for a therapeutic Oligomer conjugate. Examples of useful RNA target sequences include, the ribosome binding site, the portion of RNA coding for protein, the portion of the RNA that serves as a structural RNA and the like. Other useful target sequences include RNA sequences that when bound by an Oligomer of an Oligomer conjugate of this invention, interfere with the translation of that RNA into protein or interfere with a structural RNA's tertiary structure. Particularly preferred are Oligomer conjugates containing Oligomers that bind to a bacterial messenger RNA, a bacterial ribosomal RNA, a bacterial transfer RNA or an RNA transcript transcribed from a bacterial extrachromosomal DNA element.

In other preferred embodiments, the Oligomers in the Oligomer conjugates of the present invention are complementary to a DNA molecule present in a bacterium (DNA target sequence). The DNA present in a bacterium may be

present as part of the genome of the bacterium, the bacterial chromosome or an extrachromosomal DNA element. Many portions of a DNA molecule present in a bacterium may be selected as a DNA target sequence. Examples of useful DNA target sequences include, the origin of replication for the bacterial chromosome, the origin of replication for an extrachromosomal DNA element, a transcription initiation site, a regulator binding site, the recognition sequence of DNA binding proteins, the DNA sequences bound by RNA polymerase, the region where RNA transcription terminates and the like. Other useful DNA target sequences include DNA sequences that when bound by an Oligomer portion of an Oligomer conjugate of this invention, interfere with the transcription of a gene present in the bacterial genome, including genes present on the bacterial chromosome or on an extrachromosomal DNA element.

2. Spacers

In some embodiments, the present invention contemplates an Oligomer conjugate comprising the formula:



wherein the spacer comprises a skeleton of from about 1 to 30 atoms, wherein the Oligomer is covalently linked to the spacer and the Oligomer uptake enhancer molecule is covalently linked to a carbon atom of the spacer by preferably an O, N, or S atom. The spacer further comprises a backbone of about 10 to about 30 of a combination of carbon, nitrogen and oxygen atoms.

In other embodiments, Oligomer conjugates of the present invention have a spacer that is attached to both the Oligomer and the Oligomer uptake enhancer molecule thereby forming an Oligomer conjugate of the present invention. The general structure of this spacer is shown in Figure 2. Useful spacers contain from 2 to 30 carbon atoms and may also contain nitrogen and oxygen atoms. One skilled in the art will understand that the structure of this spacer may vary from the structure shown in Figure 2 and still be encompassed by this invention.

Spacers may be attached to an Oligomer at any number of positions, including attachments to the Oligomer via the base residue of a nucleoside, the sugar residue of a nucleoside, the 5' most nucleoside, the 3' most nucleoside, to a phosphate residue, to the 5' most phosphate group or to the 3' most oxygen atom or may comprise a non-nucleoside monomeric unit incorporated into the Oligomer. Examples of linkages at these various positions are disclosed in Goodchild, J., Bioconjugate Chemistry, Vol 1:165-187 (1990). Non-nucleoside reagents which may be incorporated into an Oligomer are described in the following PCT publications: WO 89/02933 (Arnold et al.), WO 89/02439 (Arnold et al.) and WO 92/02532 (Reynolds, et al.).

Spacers may be coupled to Oligomers either through naturally occurring sites in the Oligomer or through a reactive moiety introduced into the Oligomer for that purpose. Groups naturally present in Oligomers that are useful in coupling include amino groups on the bases, hydroxyl groups on the sugars, and phosphate groups both internal and terminal. Reactive moieties attached to the Oligomer useful for attaching a spacer to the Oligomer include primary amines, thiols and aldehydes and many other possibilities limited only by the skill in the art.

For example, a spacer or a portion of a spacer may be attached to a pyrimidine at, for example, the C5 of deoxyuracil (dU), the N4 of deoxycytosine or the C8 of an

adenine (dA). Spacers useful for attachment to the N4 of dC include $(CH_2)_6OH$ and $(CH_2)NHCO(CH_2)_5NH_2$ as respectively described in Horn, T., and Urdea, M.S., Nucleic Acids Res., 17:6959-67 (1989) and Urdea, M.S. et al., Nucleic Acids Res., 16:4937-56 (1988). Spacers useful for attachment to the C5 of dU include: $(CH_2)_3NH_2$, described by Gibson, K.J., and Benkovic, S.J., Nucleic Acids Res., 15:6455-67 (1987); $C{=}C(CH_2)_2NH_2$ described by Jablonski et al., Nucleic Acids Res., 14:6115-28 (1986). One skilled in the art will understand that if a portion of the spacer is first attached, further reactions would attach the remainder of the spacer. See, e.g., Figure 2.

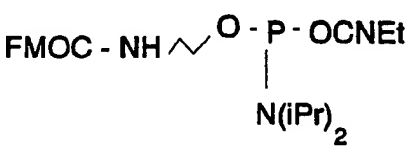
Spacers may be attached to the 5' end of an oligomer using various methods. For example, the 5'-O of the sugar may be replaced with nitrogen or sulfur allowing the resulting thiol or primary amine at the 5' end to react with an electrophile present in a spacer. These methods have been described in Smith, L.M. et al., Nucleic Acids Res., 13:2399-2412 (1985), Sproat, B.S. et al., Nucleic Acids Res., 15:4837-4848 (1987), and Sproat, B.S. et al., Nucleic Acids Res., 15:6181-6196 (1987). Other methods for attaching a spacer to the 5' end of an Oligomer include the methods described by Hemnelsbach, F., and Pfleiderer, W., Tetrahedron Lett., 23 4793-96 (1982) in which protected thymidine 3', 5' diphosphate was used to introduce a terminal 5' phosphate residue useful as a site for coupling to the spacer.

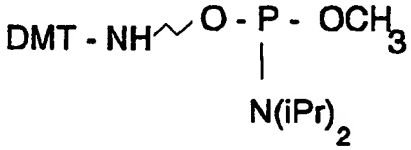
Spacers may be attached to the 3' end of an oligomer using variations of the older solution-phase triester synthesis method. Examples of spacers attached to the 3' end of an Oligomer in this manner are described by Thoung, N.T. and Chassignol, M., Tetrahedron Lett., 28:4157-60 (1987); Belikova, A.M. et al., Tetrahedron Lett., 3557-62 (1967); Francois, J.C. et al., J. Biol. Chem., 264:5891-98 (1989). Alternatively, spacers may be attached to the 3' end of an Oligomer produced by solid-phase synthesis using a special support bearing a modified nucleotide that when

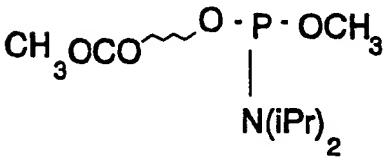
cleaved from the support provides a reactive group to which a spacer is attached. An example of this strategy includes the use of a support bearing cytidine with an amine linker at N4 described by Li, P., et al., Nucleic Acids Res., 15:5275-87 (1987).

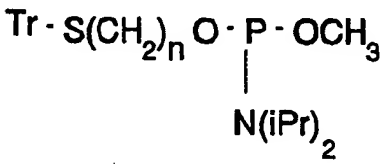
A spacer may be attached to the 5' end of an Oligomer by incorporating a non-nucleotide into the Oligomer during synthesis. These non-nucleotide reagents typically form a portion of the entire spacer and yield a nucleophilic molecule after deblocking allowing them to couple to an additional portion of a spacer or to an Oligomer uptake enhancer molecule.

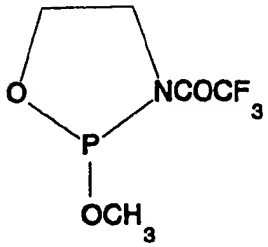
Spacers and portions of spacers useful for incorporation into an Oligomer include those that have a phophoramidite at one end separated by several carbon atoms from a protected amine, thiol or carboxyl at the other end. Examples of such spacers include:

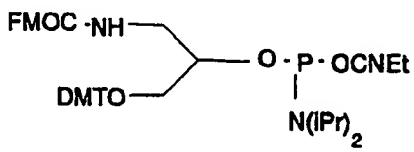








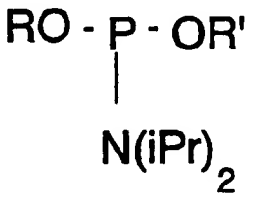


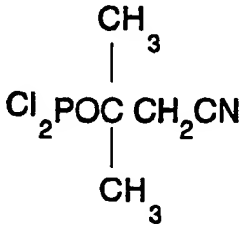


Use of these non-nucleotide reagents is described by: Agrawal, S. et al., Nucleic Acids Res., 14:6227-45 (1986); Connolly, B.A., Nucleic Acids Res., 13:4485-4502 (1985); Coull, J.M. et al., Tetrahedron Lett., 27:3991-94 (1986),

Kremsky, J.N. et al., Nucleic Acids Res., 15:2891-2909 (1987); Connolly, B.A., Nucleic Acids Res., 15:3131-39 91987); Bischoff, R. et al., Anal Biochem., 164:336-44 (1987); and Blanks, R. and McLaughlin, L.W., Nucleic Acids Res., 16:10283-99 (1988). Analogous spacers are available for H-phosphonate synthesis, triester synthesis or methylphosphonate linking and have been described by: Sinha, N.D. and Cook, R.M., Nucleic Acids Res., 16:2659-69 (1988); Ansorge, W., et al., J. Biochem. Biophys. Methods, 13:315-23 (1986); and Agrawal, S., Tetrahedron Lett., 30:7025-28 (1989).

The 5'-hydroxyl group of an oligomer may be phosphorylated or thiophosphorylated during automated synthesis using the methods described by Horn, T. Urden, M.S., DNA, 5:421-26 (1986) and Kempe, T. et al., Nucleic Acids Res., 13:45-57 (1985). This allows the phosphate to be used for attachment of a spacer or an Oligomer uptake enhancer molecule. Reagents useful for phosphorylation or thiophosphorylation include:





Any other useful chemistry for attaching a spacer to the 5'-hydroxyl include activation with carbonyldiimidazole and subsequent reaction with hexamethylenediamine to give a carbamate as described by Wachter, L. et al., Nucleic Acids Res., 14:7985-94 (1986).

A spacer may be attached to the 3' end of an oligomer during automated Oligomer synthesis by using a solid-phase support that has a modification present. The modification creates a reactive moiety after the Oligomer is cleaved from the solid support. For example, creation of a 3'-terminal thiol and a 3' terminal amine from a carbamate

useful for spacer attachment was described by Zuckerman, R. et al., Nucleic Acids Res., 15:5305-21 (1987) and by Nelson, P.S. et al., Nucleic Acids Res., 17:7187-94 (1989).

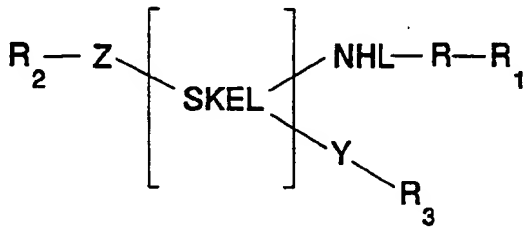
An internucleoside phosphate residue of an Oligomer may be used as a point of spacer attachment. For example, a protected amino spacer was coupled to an oligomer by Agrawal, S., and Tawy, J.-Y, Tetrahedron Lett., 31:1543-46 (1990). The spacer may also be attached to a phosphorothioate that has replaced the internucleoside phosphate by reacting the sulfur atom of the phosphorothioate with an alkylating reagent. A number of alkylating agents have been used including those described by Oshevski, S.I., FEBS Lett, 143:119-23 (1982) and Fidanza, J.A. and McLaughlin, L.W., J. Am. Chem. Soc., 111:9117-119 (1989).

Other spacers useful in practicing the present invention include the primary alkylamines and hydrazines such as various activated forms of a carboxylic acid including the ester of N-hydroxysuccinimide as described by Ghosh, S.S. and Musso, G.F., Nucleic Acids Res., 15:5353-72 (1987); nitrophenyl esters as described by Letsinger, R. L. and Schott, M.E., J. Am. Chem. Soc., 103:7394-96 91981); pentachlorophenyl esters as described by Sproat, B.S. et al., Nucleic Acids Res., 15:6181-96 (1987); acid anhydride as described by Chu, B.F. and Orgel, L.E., Proc. Nat'l Acad. Sci. U.S.A., 82:963-67 (1985); sulfonyl chlorides described by Urdea, M.S. et al., Nucleic Acids Res., 16:4937-56 91988); and the like. Other useful spacers include the isothiocyanates that form thioureas.

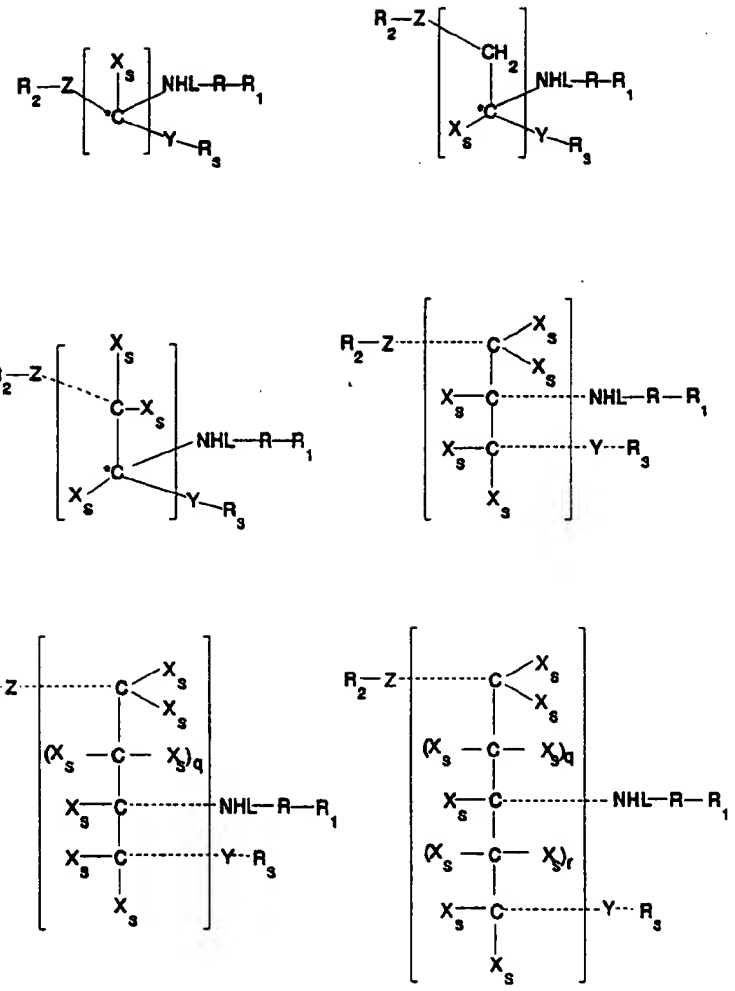
Spacers useful in practicing the present invention include thiol spacers, as described by Zuckermann, R. et al., Nucleic Acids Res., 15:5305-21 (1987). One skilled in the art will understand that the thiol chemistry is more complex than that of nitrogen spacers but that the

thiol spacers may be used in a greater variety of reactions.

According to a preferred aspect, a spacer is a non-nucleotide monomeric unit which is incorporated into the Oligomer during synthesis of the Oligomer. Useful spacers include those disclosed in Reynolds, et al., PCT Publication No. WO 92/02532 (1992) or Arnold, et al, PCT Publication No. WO 89/02439 (1989). Such spacers include non-nucleotide reagents which when incorporated into an oligomer conjugate comprise a oligomer conjugate of the formula:

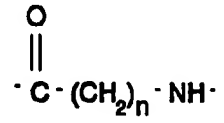


wherein SKEL comprises a non-nucleotide skeleton of from about 1 to about 20 carbon atoms, wherein -NHL, Y and Z are covalently linked to a carbon atom of SKEL, L is hydrocarbyl or a bond, Y is $-CH_2-$, -O-, -S- or -NH- and Z is -O-, -S- or -NH-; R is hydrocarbyl or a bond; R_1 is an Oligomer uptake enhancer molecule; and R_2 and R_3 are independently selected from the group consisting of a nucleoside, a nucleoside of an Oligomer, hydrogen and a blocking group. According to a preferred aspect, SKEL is chirally pure. Preferably SKEL further comprises a backbone of about 1 to about 10 carbon atoms separating Y and Z. Examples of Oligomer conjugates incorporating preferred SKEL groups include:



where the X_s groups are independently selected from hydrogen or alkyl and may be the same or different, and q and r are independently selected integers from 0 to 10.

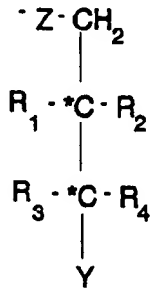
In preferred embodiments, L has the following formula:



wherein n is an integer between 1 and 15, preferably between 1 and 5.

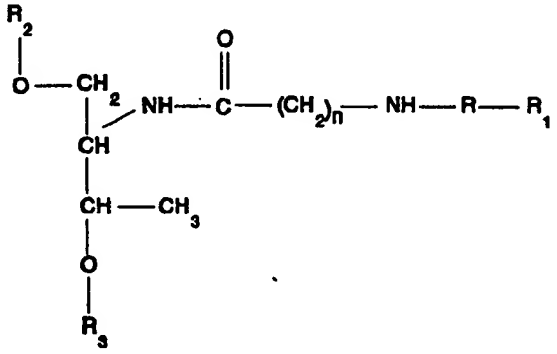
One group of especially preferred Oligomer conjugates has a skeleton derived from the amino acid threonine. These preferred skeletons comprise a 3-carbon backbone having two asymmetric carbons, each of which remains chirally pure when incorporated in a nucleotide/non-nucleotide polymer. In addition, these skeletons having threonine-derived backbones advantageously have a primary hydroxyl and a secondary hydroxyl, which due to their differing reactivities allow selectivity and high yields in the subsequent protection, deprotection, blocking, deblocking and derivatization steps.

Thus, according to an especially preferred aspect of the present invention, the threonine-based skeletons have the following formula:

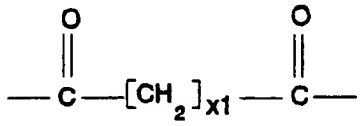


wherein *C denotes an asymmetric carbon which is chirally pure, and wherein one of R_1 and R_2 is hydrogen and the other is -NH-L-R-R_2 where L is a hydrocarbyl as hereinafter defined; one of R_3 and R_4 is hydrogen and the other is lower alkyl of about 1 to about 10 carbon atoms; and Y and Z are independently -CH_2-, -S-, -NH-, or -O-.

In certain preferred embodiments, the spacer has the structure:

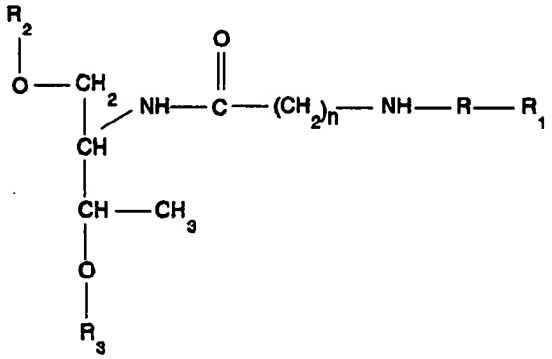


wherein n is an independently selected integer from 1 to 12, and R is



where x_1 is an integer from 1 to 12. Preferably, n is an independently selected integer from 1 to 6 and x_1 is an independently selected integer from 2 to 8. More preferred are spacers in which n is an independently selected integer from 1 to 3 and x_1 is an independently selected integer from 3 to 7. Most preferred are spacers in which n is 1 and x_1 is 6.

One skilled in the art will understand that spacers useful for linking an Oligomer to an Oligomer uptake enhancer molecule to form an Oligomer conjugate of this invention, will include spacers in which a portion of the spacer is defined by the formula:



wherein n is an independently selected integer from 1 to 12, and R is comprised of carbon and oxygen atoms having one carbon atom covalently linked to a nitrogen atom of said portion of the spacer, and a second carbon atom covalently linked to an Oligomer uptake enhancer molecule of the present invention. Useful spacers are described by Reynolds et al., PCT Publication No. WO92/02532 (1992).

More preferred are conjugates wherein n is an independently selected integer from 1 to 6; R is an

organic molecule such as a hydrocarbyl and is comprised of 1 to 20 carbon atoms; R_1 is an Oligomer uptake enhancer molecule; and R_2 and R_3 are independently selected from the following group: a nucleoside, a nucleoside that is part of an Oligomer or a Oligomer, a hydrogen, a blocking group, and the like.

3. Oligomer Uptake Enhancer Molecules

The novel Oligomer conjugates of the present invention and the Oligomer conjugates used according to the methods of the present invention include an Oligomer uptake enhancer molecule. Optionally, these Oligomer conjugates may include more than one Oligomer uptake enhancer molecule, either the same or different.

Oligomer uptake enhancer molecules include molecules which by themselves are taken up by bacteria or other cells. Such molecules include nutrients such as carbohydrates, glycerol, citrate, phosphates, sulphates, amino acids, peptides, polypeptides, proteins, polyamines, nucleic acids, lipids, siderophores and other iron-complexing agents, vitamins, and nucleosides, which enter bacterial cells by a variety of uptake systems; as described, for example, in Ames, G.F-L. (1986) Ann. Rev. Biochem. 55:397-425; Postle, K. (1990) Mol. Microbiol. 4:2019-2025; Higgins, C.F. Methods Enzymol. 125:365-377; Tabor, C.W. and Tabor, H. Microbiological Rev. 49:81-99; Crosa, J.H. (1989) Microbiol. Rev. 53:397-425. Other molecules include antibiotic substances or derivatives thereof which are known to enter bacterial cells; for example, those described in Chopra, I. and Ball, P. (1982) Adv. Micro. Phys. 23:183-240, and Livermore, D.M. (1991) Scand. J. Inf. Dis. S74:15-22.

a. Polyamines

According to one preferred aspect, the present invention contemplates Oligomer conjugates having as an Oligomer uptake enhancer molecule a polyamine that is

either covalently linked to the spacer which is in turn covalently linked to the Oligomer or is directly linked to the Oligomer. Suitable polyamines include polyamines of the formula:

$$HR_aN[(CH_2)_nNH]_m-$$

wherein R_a is alkyl of 1 to 10 carbon atoms, n is an independently selected integer between 2 and 10 and m is an independently selected integer between 1 and 10. According to more preferred embodiments, in the above formula n is an independently selected integer between 2 and 6 and m is an independently selected integer between 1 and 4. In the still more preferred embodiments, in the above formula n is an independently selected integer between 3 and 4 and m is an independently selected integer between 1 and 3.

In some preferred embodiments, the polyamine is covalently linked to a spacer and has the formula:

$$H_2N(CH_2)_{n1}NH(CH_2)_{n2}NH(CH_2)_{n3}NH-$$

wherein n1 is an independently selected integer between 3 and 4; n2 is an independently selected integer between 3 and 4 and n3 is an independently selected integer between 3 and 4. In other preferred embodiments, in the above formula n1 is 3, n2 is 4 and n3 is 3.

In other preferred embodiments, the polyamine covalently linked to the spacer has the formula:

$$NH_2(CH_2)_{n1}NH(CH_2)_{n2}NH-$$

wherein n1 is an independently selected integer between 3 and 4 and n2 is an independently selected integer between 3 and 4. In other most preferred embodiments, in the above formula n1 is 4 and n2 is 3.

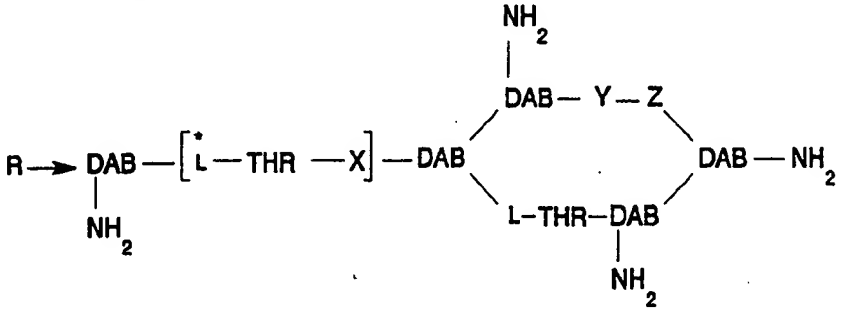
b. Membrane Disruption Molecules

According to an alternate preferred aspect, the present invention contemplates Oligomer conjugates having as an Oligomer uptake enhancer molecule a membrane disruption molecule that is either covalently linked to the spacer which is in turn covalently linked to the

Oligomer or is directly linked to the Oligomer. Suitable membrane disruption molecules include those such as the polymyxins and octapeptins shown in Table A below.

Table A

Composition of some polymyxins and octapeptins (complied from data in Parker and Rathman, 1975 and Storm, Rosenthal, and Swanson, 1971).



Antibiotic	R	X	Y	Z
Polymyxin A	MeOct	D-DAB	D-LEU	L-THR
Polymyxin B1	MeOct	L-DAB	D-PHE	L-LEU
Polymyxin B2	MeHep	L-DAB	D-PHE	L-LEU
Polymyxin D1	MeOct	D-SER	D-LEU	L-THR
Polymyxin D2	MeHep	D-SER	D-LEU	L-THR
Polymyxin E1 (Colistin A1)	MeOct	L-DAB	D-LEU	L-LEU
Polymyxin E1	MeHep	L-DAB	D-LEU	L-LEU
Circulin A	MeOct	L-DAB	D-LEU	L-ILEU
Octapeptin B1	βOHMeDec	--	D-LEU	L-LEU
Octapeptin A2	βOHMeNon	--	D-LEU	L-LEU
Octapeptin A3	βOHDec	--	D-LEU	L-LEU
Octapeptin B1	βOHMeDec	--	D-LEU	L-PHE
Octapeptin B2	βOHMeNon	--	D-LEU	L-PHE
Octapeptin B3	βOHDec	--	D-LEU	L-PHE
Octapeptin C1	βOHMeDec	--	D-PHE	L-LEU

DAB = Diaminobutyric acid
MeOct = 6-Methyloctanoic acid
MeHep = 6-Methylheptanoic acid
MeDec = 8-Methyldecanoic acid
MeNon = 8-Methylnonanoic acid
Dec = n-decanoic acid
Polymyxin B is a mixture of Polymyxins B1 and B2
Octapeptins -- omit dipeptide in square brackets

Other membrane disruption molecules include those described in Vaara, M. et al., Microbiol. Revs. 56(3); 395-411 (1992) and Gale, E.F. et al., the Molecular Basis of Antibiotin Action, John Wiley and Sons, London 1972. Examples of membrane disruption molecules useful in the present invention include: membrane-disrupting polycationic fatty acyl-containing peptides such as the polymyxins, octapeptins, brevistin, cerexin, polypeptin, stendomycin, or synthetic or semi-synthetic derivatives thereof; membrane pore-forming cyclic peptides such as the tyrocidins, valinomycin and gramicidin S; membrane pore-forming linear peptides such as gramicidins, alamethicin, and suzukacillin; other peptides reported to disrupt bacterial membrane structure and function including defensins, cecropins, magainins, melittin, bacterenectins, seminalplasmin, apidaecins, abaecins, nisin, bactericidal/permeability-increasing protein (BPI), lactoferrin, azurocidin, cathepsin G, and attacins; ionophores such as 2,4-dinitrophenol, 3,5,3',4'-tetrachlorosalicylanilide, nigericin, and monensin; agents which have been reported to disrupt the barrier function of the Gram-negative outer membrane such as polymyxin-derived peptides, polylysines and polyornithines, protamine, compound 48/80 (a polycationic polymer of the organic monovalent cation para-methoxy-phenethylmethylamine), aminoglycosides, tris(hydroxymethyl)aminomethane (TRIS), ethylenediaminetetraacetic acid (EDTA); polyenes; synthetic versions of these membrane disruption molecules; and the like. See, Hall, J.B., Biochem. Pharmacol. 32:449-453 (1983) and Vaara, Microbiol. Rev. 56(3):395-411 (September, 1992).

Selection of a membrane disruption molecule to incorporate into a particular Oligomer conjugate will depend on a number of factors including the intended target of the Oligomer conjugate, whether, for example, the target is a eukaryotic cell, a bacterial cell, a gram

negative bacterial cell, a gram positive bacterial cell, a yeast cell, a mollicute cell, a virally infected cell, or some other cell. The intended target affects the choice of membrane disruption molecules because each of these molecules is believed to have a particular spectrum of action against the various cells. The various cells are believed to have membranes composed of different constituents, different structures and to be different in a number of other parameters that alter the action of a particular membrane disruption molecule on a particular cell. Certain membrane disruption molecules are believed to be more effective against gram positive bacteria. Examples of such membrane disruptive molecules believed to be more effective against gram positive bacteria include, but are not limited to, the octapeptins, gramicidins and valinomycin. Other membrane disruptive molecules are believed to be more effective against gram negative bacteria. Examples of such membrane disruptive molecules believed to be more effective against gram negative bacteria include, but are not limited to polymyxin B, polymyxin B_1 and polymyxin B_2.

The spectrum of action of certain membrane disruption molecules is described in Vaara, M. et al., Microbiol. Revs. 56(3):395-411 (1992) and Gale, E.F. et al., The Molecular Basis of Antibiotic Action, John Wiley and Sons, London 1972.

C. Compositions Containing an Oligomer Conjugate

According to one preferred embodiment, this invention provides a composition comprising a pharmaceutically acceptable and therapeutically effective amount of a substantially pure Oligomer conjugate having an Oligomer capable of hybridizing to a nucleic acid of a bacteria.

A therapeutically effective amount of an Oligomer conjugate is an amount that when administered to an animal is capable of inhibiting the growth of bacteria already present in the animal. In a prophylactic context, a

therapeutically effective amount of an Oligomer conjugate would also be sufficient to prevent a particular bacteria from infecting an animal. Assays for detecting the presence of bacteria in an animal or the inhibition of growth of bacteria in an animal are well known in the clinical arts. For example, the blood of the animal may be cultured under conditions allowing the detection of bacteria, antibodies may be used to detect the presence of bacteria in a clinical sample isolated from the animal, nucleic acid probes may be used to detect the presence of bacteria in a clinical sample from the animal, various blood chemistry and other clinical indicators of a bacterial infection may be monitored or the distribution of various types of blood cells in the circulation of the animal may be monitored (differential count). In some embodiments, in which a composition containing an Oligomer conjugate is used to treat a bacterial infection in an animal, a therapeutically effective amount of an Oligomer conjugate would at least prevent the number of bacteria present in the animal from increasing. In other embodiments, in which a composition containing an Oligomer conjugate is used to prevent a bacterial infection in an animal, a therapeutically effective amount of an Oligomer conjugate would prevent any bacteria, above a background number, from being detected in the animal.

Substantially pure, when used in the context of an Oligomer conjugate refers to compositions, such as solutions, that are enriched in the Oligomer conjugate, and preferably are substantially free of detectable amounts of free Oligomer, free Oligomer uptake enhancer molecule, and other incomplete reaction products. Preferred substantially pure Oligomer conjugates are in excess of 90 percent pure by weight of the Oligomer conjugate per total mass of Oligomer material contained in the solution or composition.

A pharmaceutically acceptable Oligomer conjugate, when used in a therapeutic composition, does not cause any

undesirable physiological effects due to the presence of contaminants. Thus, a pharmaceutically acceptable Oligomer conjugate is free of pharmaceutically unacceptable contaminants such as pyrogens (lipopolysaccharides) and other contaminants such as poisonous chemicals (i.e., sodium azide) and detergents, such as sodium dodecyl sulfate.

The preparation of therapeutic compositions which contain compounds such as Oligomer conjugates may be performed as follows. Such compositions may be prepared as injectables, either as liquid solutions or suspensions; however, solid forms suitable for dissolving in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with inorganic and/or organic excipients which are pharmaceutically acceptable and compatible with the active ingredient (Oligomer conjugate). Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents which enhance the effectiveness of the active ingredient. In any case, the administered composition contains at least about 0.001% to about 99% by weight of an Oligomer conjugate per unit weight of composition, preferably 0.005% to 1.0% for an IV solution, and preferably 10 to 90% for a solid, more preferably 25 to 75%.

A therapeutic composition useful in the practice of the present invention typically contains an Oligomer conjugate formulated into the therapeutic composition as a neutralized pharmaceutically acceptable salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with any free amino groups of the Oligomer conjugate) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric

acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

In the preferred embodiments, a therapeutic composition of the present invention contains a therapeutically effective amount of an Oligomer conjugate and is a sterile composition. A sterile composition is substantially free of bacteria and fungus. Typically a composition is made sterile by passing the composition through a filter such as a 0.2 micron filter designed for this purpose.

In other preferred embodiments, a composition of the present invention is optimized to allow the Oligomer conjugate it contains to be delivered transdermally or directly to a selected tissue. Methods for transdermal delivery of a drug or delivery to a specific tissue are well known in the art. See, for example, Arnold and Maxwell, PCT Publication No. WO 92/21353 (December 10, 1992); Zaffaroni, U.S. Patents 4,186,184 and 3,948,254; Eppstein et al., U.S. Patent 4,962,091; and Chiang et al., U.S. Patent 4,973,468 the disclosures of which are incorporated herein by reference.

D. Methods Of Enhancing the Uptake of an Oligomer Into A Bacterial Cell Using An Oligomer Conjugate

The present invention also contemplates methods of using the Oligomer conjugates of this invention to treat or prevent a bacterial infection in an organism such as an animal. Generally, the method comprises administering to an organism an Oligomer conjugate of the present invention in an amount sufficient to establish in the organism a therapeutic concentration of the Oligomer conjugate.

Oligomer conjugates useful in the practice of the methods of the present invention include Oligomer conjugates having an Oligomer directly coupled to an Oligomer uptake enhancer molecule. Oligomer conjugates having an Oligomer coupled to a spacer that is coupled to an Oligomer uptake enhancer molecule are also useful in practicing the methods of the present invention. Thus, in the methods of the present invention in the Oligomer conjugate present, the Oligomer and Oligomer uptake enhancer molecule may be directly linked together or when a spacer is present, the Oligomer and Oligomer uptake enhancer molecule are both linked to a spacer. The Oligomer conjugate typically contains an Oligomer that is between about 6 and about fifty nucleoside units in length. In more preferred embodiments, the Oligomer present as part of the Oligomer conjugate is from about 8 to about 30 nucleotide units in length.

In several embodiments of this invention, Oligomer conjugates are used according to methods of the present invention to obtain increased uptake in bacterial cells are contemplated that contain an Oligomer uptake enhancer molecule and an Oligomer directly coupled together and thus do not have a spacer. Use of such Oligomer conjugates according to our methods shows an enhanced rate of Oligomer conjugate uptake into bacterial cells. These Oligomer conjugates are useful in that the coupling of the Oligomer uptake enhancer to the Oligomer unexpectedly increases the rate of uptake of that Oligomer conjugate into bacterial cells. Specifically, the methods of introducing an Oligomer into a bacterial cell as part of an Oligomer conjugate containing an Oligomer uptake enhancer molecule can be practiced using an Oligomer conjugate that lacks a spacer.

In such Oligomer conjugates that contain an Oligomer and an Oligomer uptake enhancer molecule but lack a spacer, the Oligomer and Oligomer uptake enhancer molecule are directly coupled together. One skilled in the art will understand that the Oligomer and Oligomer uptake enhancer molecule may be directly coupled using a variety

of covalent linkages. For example, the Oligomer uptake enhancer molecule may be coupled via a phosphodiester linkage to the sugar moiety or may be coupled directly to the 5' position of the sugar moiety of a nucleoside present in the Oligomer. The chemistries and techniques for directly coupling compounds to an Oligomers are well known and include, for example, the methods disclosed in Lemitre et al., Nucleosides & Nucleotides 311-315 (1987); Miller et al., Nucleosides & Nucleotides 37-46 (1991); and U.S. Patent No. 5,138,045 (1992).

This Oligomer conjugate is typically introduced into an organism, such as an animal, as a composition containing an Oligomer conjugate of the present invention dispersed in a pharmaceutically acceptable excipient. The Oligomer conjugate will typically contain an Oligomer capable of binding to (hybridizing to) a nucleic acid or protein present in a bacterium that has or might infect the organism being treated.

In preferred embodiments, the Oligomer conjugate administered to the organism has a nucleotide sequence capable of binding to a RNA transcript, messenger RNA, ribosomal RNA, transfer RNA, the genome, an extrachromosomal DNA element, the chromosome of a bacterium, a protein, a regulatory protein, a nucleic acid binding protein, an enzyme, or a nucleic acid synthesis enzyme. In preferred embodiments, the particular bacteria that are the target of the Oligomer conjugate are Gram-negative bacteria such as those listed in Section B of the Detailed Description of the invention. Alternatively, the bacteria may be a gram-positive bacteria also as listed in Section B hereinabove.

The treatment of an organism for a bacterial infection may be monitored using methods well known to those skilled in the clinical arts. For example, the blood of an animal may be cultured under conditions allowing the detection of bacteria, antibodies may be used to detect the presence of bacteria in a clinical sample isolated from the animal, nucleic acid probes may be used to detect the presence of bacteria in a clinical sample

from the animal, various blood chemistry and other clinical indications of a bacterial infection may be monitored or the distribution of various types of blood cells in the circulation of the animal may be monitored (differential count).

Organisms that can be treated with an Oligomer conjugate of the present invention include animals, plants, lower plants, higher plants, invertebrates, vertebrates, osteichthytes, chondrichthytes, amphibians, reptiles, birds (Aves), mammals, marsupials and monotremes. Animals in which the treatment or prevention of a bacterial infection would be clinically useful include animals and patients with a detectable bacteria in the blood (bacteremia) or other clinical sample, immunosuppressed animals and patients, animals or patients known to be exposed to certain bacteria and the like. The Oligomer conjugates of the present invention can be used in any animal that would benefit from treatment with a classical antibiotic and in animals in which a classical antibiotic would be ineffective. Classical antibiotics would be clinically not indicated in some situations because, for example, of antibiotic resistance, allergies and the like.

An Oligomer conjugate of the present invention is typically administered as a pharmaceutically acceptable composition in the form of a solution or suspension. However, as is well known, Oligomer conjugates can also be formulated for therapeutic administration as tablets, pills, capsules, sustained release formulations or powder, or formulated for transdermal administration. Oligomer conjugates may also be administered as a solid, a liquid or a paste.

The composition is administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's blood hemostatic system to utilize the active

ingredient, and degree of either the inhibition of growth of a bacteria already present in the animal or the amount of preventive therapy desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

A therapeutically effective amount of an Oligomer conjugate can be expressed as an amount sufficient to produce a final concentration of an Oligomer conjugate in a patient's blood. That blood concentration can be determined by an in vitro assay for the Oligomer conjugate in a liquid body sample (e.g., blood), as is well known, or can be calculated based on the patient's body weight and blood volume, as is well known.

Suitable dosage ranges of an Oligomer conjugate for the therapeutic methods described herein are in the order of 0.1 to 250 mg, preferably 1 to 100 mg, more preferably 1 to 10 mg of Oligomer conjugate per kilogram of body weight of patient per day, and depending on the route of administration. Stated differently, a therapeutically effective dosage is an amount sufficient to produce an intravascular concentration of Oligomer conjugate in the blood of the patient in the range of 0.1 to 100 μg/ml, and preferably about 10 to 20 μg/ml. The therapeutic effective amounts of Oligomer conjugates typically produce intracellular concentrations of an Oligomer of about 0.01 μM to about 30 μM. More preferably, the intracellular Oligomer concentrations produced are from about 0.1 μM to about 20 μM. Most preferred are intracellular Oligomer concentrations of about 0.1 μM to about 3 μM.

The therapeutic composition containing an Oligomer conjugate is conventionally administered parenterally, as by injection of a unit dose. Injection may be intravenous, intramuscular, intraperitoneal or subcutaneous. However, delivery of an Oligomer conjugate containing composition transdermally or orally is also contemplated. The term "unit dose," when used in refer-

ence to a therapeutic composition used in the present invention, refers to physically discrete units suitable as unitary dosages for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required excipient.

In other preferred embodiments, an Oligomer conjugate of the present invention can be administered and is therapeutically effective through buccal, nasal, colonic and rectal mucosa using various approaches. See, Eppstein, et al., Alternative Delivery Systems for Peptides and Proteins as Drugs, CRC Critical Rev. in Therapeutic Drug Carrier Systems, 5:99-139 (1988).

In other preferred embodiments, the Oligomer conjugate is administered by intranasal formulations in solution. The formulation is administered by one of three ways: a single dose through a catheter; multiple doses through metered dose pumps (also called nebulizers); and multiple doses through the use of metered dose pressurized aerosols. If desired, the absorption of the Oligomer conjugate across the nasal mucosa may be promoted by adding absorption enhancers including nonionic polyoxyethylene ethers, bile salts such as sodium glycocholate (SGC) and deoxycholate (DOC), and derivatives of fusidic acid such as sodium taurodihydrofusidate (STDHF). Dosages of Oligomer conjugates can range from 0.15 mg/kg up to 600 mg/kg; preferred dosages range from 0.15 mg/kg up to 200 mg/kg; and most preferred dosages range from 0.15 mg/kg up to 10 mg/kg in a nasal spray formulation.

Several dosage forms are available for the rectal delivery of Oligomer conjugates. These include suppositories (emulsion and suspension types), rectal gelatin capsules (solutions and suspensions), and enemas (macro: 100 ml or more; and micro: 1 to 20 ml). Osmotic pumps designed to deliver a volume of 2 ml in a 24 to 40 hour period have also been developed for rectal delivery. Absorption enhancers described for nasal formulations are

included in the formulations if increased transport across rectal mucosa is desired. A preferred formulation for rectal administration of the Oligomer conjugate consists of the preferred ranges listed above in any one of the acceptable dosage forms.

Oligomer conjugates can be administered in a liposome (micelle) formulation which can be administered by application to mucous membranes of body cavities. See, Juliano et al., J. Pharmacol. Exp. Ther., 214:381 (1980). Liposomes are prepared by a variety of techniques well known to those skilled in the art to yield several different physical structures, ranging from the smallest unilamellar vesicles of approximately 20 to 50 nanometers in diameter up to multilamellar vesicles of tens of microns in diameter. Gregoriadias, Ed., Liposome Technology, 1 CRC Press (1984). The Oligomer conjugates in the preferred dosages listed for nasal formulations are hydrated with a lyophilized powder of multilamellar vesicles to form Oligomer conjugate-containing liposomes.

In other embodiments, the present invention contemplates treating or preventing a bacterial infection in an animal according to the method having the following elements:

1. Administering to an animal, such as a mammal, an Oligomer conjugate of the present invention in an amount sufficient to establish within the animal a therapeutic concentration of the Oligomer conjugate; and

2. Maintaining the therapeutic amount of Oligomer conjugate within the animal for a predetermined time period sufficient to treat the animal.

In other embodiments, the present invention contemplates preventing a bacterial infection in an animal, such as a mammal, according to the method having the following elements:

1. Administering to an animal an Oligomer conjugate of the present invention in an amount sufficient to

establish within the animal a prophylactic concentration of the Oligomer conjugate; and

2. Maintaining the prophylactic concentration of Oligomer conjugate within the animal for a predetermined time period sufficient to prevent a bacterial infection in the animal.

A prophylactic concentration of an Oligomer conjugate is a concentration such that an animal exposed to bacteria does not become infected with that bacteria. In preferred embodiments the prophylactic concentration of Oligomer conjugate is sufficient to prevent the animal from becoming infected or colonized with the bacteria. When an Oligomer is used prophylactically, the Oligomer conjugate or a composition containing the Oligomer conjugate is administered to an organism that is not already infected with bacteria to increase that organism's resistance to infection by bacteria. The precise amounts of Oligomer conjugate used depend on the organism's health and general level of immunity. Suitable prophylactic dosages of an Oligomer conjugate are generally in the order of 0.01 to 25 mg per kilogram of body weight per day.

The amount of time that treatment must be continued to arrest, eliminate or prevent a bacterial infection is well known in the clinical arts. Preferred time periods are those time periods sufficient to make sure that enough bacteria in the animal are killed to prevent reinfection from bacteria still present in the animal after the treatment is discontinued. Typical preferred predetermined time periods are from about 1 hour to about a year, more preferred are predetermined time periods of about 1 day to about 30 days and most preferred are predetermined time periods of about 3 days to about 14 days.

The present invention also contemplates introducing an Oligomer conjugate into a bacterial cell according to the method having the following elements:

1. Contacting an Oligomer conjugate of the present invention with a bacterial cell under intracellular accumulation conditions; and

2. Maintaining the contact between the Oligomer conjugate and the bacterial cell under intracellular accumulation conditions for a predetermined time suffi-cient for the Oligomer conjugate to enter the bacterial cell.

The Oligomer conjugate may be contacted with the bacteria in a variety of ways. For example, a composition containing the Oligomer conjugate could be admixed with bacteria or a composition containing bacteria. Compositions containing bacteria include body fluids such as blood or serum that contain bacteria, foods containing bacteria, solutions containing bacteria and the like. The Oligomer conjugate could also be present as a solid or paste. For example, solid or paste Oligomer conjugates could be embedded in small cavities in a surface and then that surface could be contacted with a solution containing a bacteria.

The Oligomer conjugates useful for introduction into bacterial cells via this method include any of the Oligomer conjugates described in this patent application including those Oligomer conjugates having an Oligomer directly attached to a polyamine and those Oligomer conjugates having an Oligomer attached to a spacer which is also attached to a polyamine. The Oligomer conjugates preferred for introduction into a bacteria contain an Oligomer that is capable of binding to a nucleic acid or protein present in the bacteria. Typical useful Oligomer are those that bind RNA, DNA, a regulatory protein or an enzyme inside the bacteria.

The contact between the bacteria and the Oligomer conjugate is maintained for a time period that has been predetermined and is sufficient for the Oligomer conjugate to enter the cell. Predetermined time periods sufficient to allow the Oligomer conjugate to enter the cell include time periods from about one minute to five days.

Preferred predetermined time periods sufficient to allow an Oligomer conjugate include from about one hour to about 24 hours. Most preferred are predetermined time periods from about six hours to about 22 hours.

Intracellular accumulation conditions are conditions of temperature, pressure, osmolality, pH and Oligomer conjugate concentration such that the Oligomer conjugate will accumulate inside the bacteria cell at a faster rate than the accumulation rate of an unconjugated Oligomer. Typical intracellular accumulation conditions include physiologic conditions, bacterial cell culture conditions, mammalian cell conditions, condition similar to the serum of an animal, plant culture conditions and the like.

Physiologic conditions are those conditions of temperature, pressure, osmolality and pH that are compatible with living organisms. Due to the robust nature of bacteria, physiologic conditions include temperatures from 0°C to about 90°C, pressures from about 0.5 atmosphere to about 5 atmospheres, pH from about 0 to about 10.0 and osmolality of water to solutions saturated with salts. Bacterial cell culture conditions are conditions under which bacteria can be grown and are well-known in the art. Mammalian cell culture conditions are conditions under which mammalian cells or mammalian tissues can be grown and these conditions are well known in the art. Plant culture conditions are those conditions under which plants or plant cells can grow and these conditions are also well known.

Other important clinical methods contemplated by the present invention include any clinical application that currently employs a classical antibiotic to prevent or treat a bacterial infection, including in tissue culture, agriculture, foods, the environment, and the like. For

example, the present invention contemplates disinfecting an implantable medical device according to the method having the following elements:

1. Contacting an Oligomer conjugate of the present invention with an implantable medical device; and

2. Maintaining the contact between the Oligomer conjugate and the implantable medical device for a pre-determined time period sufficient to disinfect the implantable medical device.

Implantable medical devices are well known in the art and include devices such as catheters, intravenous injectors, tubes, pumps, artificial joints, artificial organs, electrodes, electronic devices and the like. These implantable medical devices are typically placed in the body using standard surgical techniques. The contact between the implantable medical device and the Oligomer conjugate may be at the surface, in cavities in the surface, below the surface or on an internal surface of the device. The Oligomer conjugate may be formulated so that it is actually manufactured into the device.

E. Therapeutic Agents for Inhibiting the Growth of Bacteria

The present invention contemplates therapeutic agents for inhibiting the growth of a bacteria with an Oligomer conjugate containing an Oligomer uptake enhancer molecule attached directly or indirectly to an Oligomer wherein a portion of its nucleic acid residue sequence is capable of interacting with (binding to) a nucleic acid or a protein present on or inside the bacteria. The Oligomer may contain a nucleic acid residue sequence that is substantially complementary to a nucleic acid in the bacterial cell. The binding of this bacterial nucleic acid to the Oligomer inhibits the function of the bacterial nucleic acid in replication, transcription, translation, regulation and the like.

In other embodiments, the Oligomer conjugate present as part of the therapeutic agent contains an Oligomer having a nucleic acid residue sequence that is substantially similar to the nucleic acid residue sequence that is bound by a protein in a bacterial cell. The protein typically binds a specific nucleic acid residue sequence and this interaction affects the growth of the bacteria. The Oligomer interferes with this nucleic acid protein interaction and thus affects bacterial growth.

These therapeutic agents contain Oligomer conjugates of the present invention that have an Oligomer uptake enhancer molecule directly or indirectly attached to an Oligomer. When the Oligomer and Oligomer uptake enhancer molecule are indirectly linked, this linkage is typically provided by a spacer of the present invention. The therapeutic agents contemplated by the present invention may also contain multiple Oligomers, spacers and Oligomer uptake enhancer molecules in various ratios as described elsewhere in this disclosure.

A target nucleic acid is a nucleic acid that contains a nucleic acid residue sequence that is bound by a bacterial protein or is bound by another nucleic acid containing a nucleic acid residue sequence that is substantially complementary to the target nucleic acid. A target nucleic acid sequence is the portion of a target nucleic acid that is bound by either a portion or another nucleic acid.

Examples

The following examples are intended to illustrate, but not limit, the scope of the invention.

1. Preparation of an Radioactive Oligomer Conjugate

(a) Preparation of an Oligomer-MP containing a C_2-Spacer

A methylphosphonate Oligomer having a C_2 spacer was synthesized using the methods disclosed in Reynolds et al., Int'l Publication No. WO 92/02532, published

February 20, 1992. The synthesis procedure is briefly described below:

(i) Reduction of L-Threonine Methyl Ester

L-Threonine methyl ester (purchased from Sigma) was reduced according to the procedure of Stanfield et al., J. Org. Chem., 46:4799 (1981); in a 500 ml three necked flask, 5 g of L-threonine methyl ester and 200 ml dry THF were mixed and 150 ml of 1 M solution of $LiAlH_4$ was added dropwise with stirring while under argon. The reaction mixture was then warmed up to the boiling temperature of THF and refluxed under argon overnight. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel which was visualized with ninhydrin. The reaction mixture was cooled at 5-10°C and quenched with dropwise addition of 0.25 M NaOH (100 ml). The mixture was evaporated to remove over 90% of THF and the residue was diluted with 100 ml of dimethylformamide which facilitates the filtration. The mixture was then filtered through a Whatman #1 paper using aspirator vacuum. The filtrate was evaporated to dryness and the residue was purified on a flash silica gel column. The column was packed with dichloromethane and the product was eluted with 50% methanol in dichloromethane.

(ii) Coupling of Reduced L-Threonine to the Spacer

The spacer, Fmoc-glycine (C2) (11 mmol) was dried by evaporation with pyridine (3 x 20 ml). The dry residue was dissolved in 40 ml of a mixture of anhydrous dimethylformamide and anhydrous tetrahydrofuran (1:1). The solution was cooled in an ice bath and 1 equivalent of diisopropylethylamine was added. While stirring, 1:1 equivalent of trimethylacetyl chloride was added dropwise and stirred for 45 min. at 0°C. A solution of 1.5 equivalent of reduced L-threonine (Example 1(a)(i) above) was added and the reaction mixture was allowed to warm to room temperature and stirred for one hour. The progress of the reaction was monitored by TLC on silica gel which was developed by $CH_2Cl_2/CH_3OH/CH_3COOH$ (10:1:0.1) solvent

system. After the completion of the reaction, the solvent was removed under reduced pressure and the residue was mixed with 50 ml ethyl acetate. The water soluble materials were removed by extraction with 40 ml saturated sodium bicarbonate. The organic phase was washed with 20 ml of water and dried with $MgSO_4$. The product was crystallized from ethyl acetate.

C2 Linker. ^1H NMR in DMSO-d6, 1.03 (CH_3 of reduced L-threonine), 3.35 (OH), 3.3-3.45 (2CH), 3.91 (NH), 4.27 (other NH), 4.31 (OH), 4.34 (CH_2), 4.63 (CH_2 and CH of FMOC), 7.3-7.9 (8-Aromatic protons).

(iii) Dimethoxy Tritylation of the Primary Hydroxyl Moiety of the Non-Nucleotide Reagent

The desired non-nucleotide reagent (6 mmol), which was the product of Example 1(a)(ii) above, was dried by co-evaporation with dry pyridine and dissolved in 15 ml of dry pyridine. A solution of 2.2 g of dimethoxytrityl chloride in 20 ml of CH_2Cl_2/pyridine (1:1) was added dropwise with stirring. The reaction continued at room temperature for 45 min. The progress of the reaction was monitored by TLC. After the completion of the reaction, it was quenched by the addition of 2 ml methanol which was stirred for 10 min. The solvents were removed under reduced pressure and the residue was dissolved in 50 ml of dichloromethane and extracted with saturated sodium hydrogen carbonate (2 x 50 ml) followed by water (30 ml). The organic phase was dried with $MgSO_4$ and filtered. After the evaporation of the solvent, the residue was purified with flash column chromatography. The product was eluted with 2% methanol in dichloromethane containing 0.5% triethylamine.

^1H NMR, $CDCl_3$, 1.18 (CH_3 of reduced L-threonine), 3.78 (2 CH_3's of DMT), 4.35 (CH_2-O-DMT), 5.98 (NH) 6.80-7.78 (21 aromatic protons).

(iv) Methylphosphinylation of the Secondary Hydroxyl Moiety of the Non-Nucleotide Reagents

A DMT blocked linker arm made according to the procedure described in Example 1(a)(iv) above (4 mmol) was dried by co-evaporation with dry pyridine and the residue was dissolved in 20 ml of anhydrous dichloromethane. Under closed argon atmosphere, 1.5 equivalent of diisopropylethylamine was added and 1.2 equivalent of N,N-diisopropylmethylphosphinamidic chloride $[(CH_3)_2CH]_2NP(CH_3)Cl$ was added dropwise. The reaction was completed in 45 min. The solvent was removed under reduced pressure and the residue was purified on a flash silica gel column. The column was packed with ethyl acetate/hexane (1:1) containing 5% triethylamine and washed with the ethyl acetate/hexane containing 1% triethylamine. The reaction mixture was then loaded on the column and the product was eluted with ethyl acetate/hexane (1:1) containing 1% triethylamine.

Other non-nucleotide reagents are prepared by coupling of the linker arm-modified reagents made according to the methods described in Example 1(a)(iv) with other phosphorylating agents such as N,N-diisopropylmethyl phosphonamidic chloride, $[(CH_3)_2CH]_2NP(OCH_3)Cl$, and 2-cyano-ethyl N,N-diisopropylchlorophosphoramidite, $[(CH_3)CH]_2NP(Cl)OCH_2CH_2CN]$. Such reagents are useful in the synthesis of phosphate diester coupled non-nucleotide-Oligomers.

(v) Preparation of Methylphosphonate Oligomers Which Incorporate Non-Nucleotide Reagents

(1) Preparation of Methylphosphonate Oligomers

Methylphosphonate Oligomers which incorporated non-nucleotide reagents of the present invention were synthesized using methylphosphonamidite monomers and non-nucleotide methylphosphonamidite non-nucleotide reagents, according to chemical methods described by P.S. Miller et al. (1983, Nucleic Acids Res., 11, pp. 6225-6242),

A. Jager and J. Engels (1984, Tetrahedron Lett., 25, pp. 1437-1440), and M.A. Dorman et al. (1984, Tetrahedron, 40, pp. 95-102). To allow quantification of the resulting Oligomers in biological systems, the Oligomers were radio labelled by incorporating one or more ^{3}H-methylphosphonamidite monomers which were tritiated by Moravek Biochemicals, Brea, California. Solid-phase synthesis was performed on a Biosearch Model 8750 DNA Synthesizer according to the manufacturer's recommendations with the following modifications: "G" and "C" monomers were dissolved in 1:1 acetonitrile/dichloromethane at a concentration of 100 mM. "A" and "T" monomers were dissolved in acetonitrile at a concentration of 100 mM. Non-nucleotide linker reagents were dissolved in acetonitrile at a concentration of 120 mM. DEBLOCK reagent = 2.5% dichloroacetic acid in dichloromethane. OXIDIZER reagent = 25 g/L iodine in 2.5% water, 25%, 2,6-lutidine, 72.5% tetrahydrofuran. CAP A = 10% acetic anhydride in acetonitrile. CAP B = 0.625% N,N-dimethylaminopyridine in pyridine. The 5'-dimethoxytityl protecting group was removed at the end of the synthesis.

The crude, protected non-nucleotide reagent incorporating methylphosphonate Oligomers were removed from the solid support an deprotected by first adding concentrated ammonium hydroxide (10% in 1:1 ACN/Ethanol) for 0.5 hours at room temperature. The deprotection was completed by the addition of one volume ethylenediamine and stirring at room temperature for 6 hours. The resulting solution was drained from the support and the support was washed 3 times with solution containing acetonitrile and water in equal amounts. The wash solution was added to the Oligomer solution and water was added to the solution until the organic content was about 10%. The solution was neutralized with 6 N HCL and purified as follows.

(2) Purification of Linker-Modified Methyl-Phosphonate Oligomers

The 5'-dimethoxytrityl (trityl) containing Oligomers were purified from non-tritylated failure sequences using a Sep-Pak™ C18 cartridge (Millipore/Waters, Bedford, MA) as follows: The cartridge was washed with acetonitrile, 50% acetonitrile in 100 mM triethylammonium bicarbonate (TEAB, pH 7.5), and 25 mM TEAB. Next, the crude methylphosphonate Oligomer solution passed through the cartridge. The cartridge was then washed with 25 mM TEAB to elute the salts and the failure sequences from the cartridge. Finally, the Oligomer was eluted from the cartridge with 50% acetonitrile/water and evaporated to dryness under vacuum at room temperature.

The linker-modified methylphosphonate Oligomers obtained from the previous step, above, were further purified by reverse-phase HPLC chromatography as follows: a Beckman System Gold HPLC, described in a previous example, was used with a Hamilton PRP-1 column (Reno, Nevada, 10 μ, 7 mm i.d. x 305 mm long). Buffer A = 50 mM triethylammonium acetate (pH 7); Buffer B = 50% acetonitrile in 50 mM triethylammonium acetate (pH 7). The sample, dissolved in a small volume of 10-50% acetonitrile/water, was loaded onto the column while flowing at 2.5-3 ml/minute with 100% Buffer A. Next, a linear gradient of 0-70% Buffer B was run over 30-50 min. at a flow rate of 2.5-3 ml/minute. Fractions containing full-length non-nucleotide reagent incorporating methyl-phosphonate Oligomer were evaporated under vacuum and resuspended in 50% acetonitrile/water.

(b) Coupling Disuccinimidyl Suberate to the C_2 Spacer-Methylphosphonate Oligomer

Thirty milligrams (mg) of disuccinimidyl suberate (DDS) was dissolved in 800 microliters (μl) of dimethyl sulfoxide (DMSO) in a glass vial to form a DSS solution.

Three mg of the methylphosphonate Oligomer containing the C_2 spacer that was prepared in Example 1(a) was dissolved in 50 μl of a 1 molar (M) solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) at pH 8.0 and 50 μl of DMSO to form a C_2 spacer-Oligomer solution.

The C_2 spacer-Oligomer solution and the DSS solution were cooled to 5° centigrade (C) and the solutions mixed to form a reaction admixture. The reaction admixture was stirred thoroughly and then placed on a rotary mixer for 2 hours at room temperature to allow the DSS-C_2 spacer-Oligomer reaction product to form.

A desalting column was prepared by swelling Sephadex LH-20 resin in a mixture of equal parts of acetonitrile (ACN) and water. The swollen resin was washed three times with a solution containing equal parts of ACN and water. The resin was then packed into a 1 x 30 centimeter (cm) column.

The reaction admixture was desalted by applying the reaction admixture to the desalting column and then eluting the DSS-C_2 spacer-Oligomer with a solution containing equal parts of ACN and water, while collecting 1 ml fractions in micro centrifuge tubes.

The fractions containing the DSS-C_2 spacer-Oligomer were identified by placing a small sample of each fraction on a silica-gel 60-F254 (EM Science, Cherry Hill, New Jersey) using a capillary tube, and then the samples were subjected to ultraviolet (UV) light. In samples containing the reaction product, the DSS-C_2 spacer-Oligomer, appeared as a dark center dot surrounded by a pale halo, whereas samples containing the slower eluting HEPES appeared as a uniform dot that did not have a dark center.

The samples containing the DSS-C_2 spacer-Oligomer were pooled and evaporated to dryness under a vacuum using a speed-vac apparatus. The dried DSS-C_2 spacer-Oligomer was then coupled to spermidine according to Example 1(c), below.

(c) Conjugation of Spermidine to the DSS-C_2 Spacer-Oligomer

A solution of 1 M HEPES in water was prepared and it had an unadjusted pH of 5.6. Thirty mg of spermidine was dissolved in 500 ml of the 1 M HEPES pH 5.6. solution and this resulted in a spermidine solution having a pH of 8.0. Ninety optical density units (OD's) of the DSS-C_2 spacer-Oligomer prepared in Example 1(b) were dissolved in 500 μl of dimethyl sulfoxide (DMSO) in a glass vial to form an Oligomer solution. The spermidine solution and the Oligomer solution were cooled to 5°C and then the Oligomer solution was admixed with the spermidine solution to form a reaction admixture. The reaction admixture was mixed thoroughly and stirred overnight at room temperature to allow the Oligomer conjugate to form.

The reaction admixture was desalted on a sephadex LH-20 column as described in Example 1(b) above. The resulting fractions were tested according to the procedure described in Example 1(b) and the fractions containing the Oligomer conjugate were pooled and evaporated to dryness under a vacuum using a speed-vac apparatus.

The Oligomer conjugate was then analyzed using high pressure liquid chromatography (HPLC) and then purified using a reverse phase-column as follows. The Oligomer conjugate prepared above, was dissolved in 200 microliters (μl) in a solution of equal parts acetonitrile and water to form an Oligomer conjugate solution. An HPLC apparatus having a Beckman 126 pump and a Beckman 168 diode array detector was equipped with a Whatman Partisil5 ODS3 analytical column. The Oligomer conjugate solution was injected into the column using a Beckman 507 autosampler with a flow rate of 1 ml/minute. The elution program used is shown in Table 1.

Table 1

Time*	%A*	%B*	Flow rate**	Duration[I]
0	100	0	1.0	initial
0	83	17	1.0	2
2	70	30	1.0	30
32	50	50	1.0	5
47	100	0	1.0	5

* Solution A consisted of 50 millimolar triethylammonium acetate with 1% acetonitrile.

* The time is listed in minutes.

** ml/min

I minutes

Using the elution program of Table 1, the C_2 spacer-Oligomer eluted first at around 20 min. The DSS-C_2 spacer-Oligomer eluted next at around 24 minutes into the elution program. The purity of the Oligomer conjugate was confirmed by standard techniques using electrospray ionization mass spectroscopy.

2. The Oligomer Conjugate Exhibits Increased Entry Into Bacterial Cells

(a) Preparation of Bacterial Cells for the Oligomer Conjugate Uptake Assay

Oligomer conjugates containing an Oligomer having the nucleotide sequences shown in Table 2 were used in the uptake assays. Approximately 20 nanograms (ng) of the Oligomer conjugate prepared as described in Example C were resuspended in 2 ml of Luria-Bertaini (LB) medium consisting of 10 grams per liter (g/l) Bacto-tryptone, 5 g/l of Bacto-yeast extract, 10 g/l sodium chloride and adjusted to pH 7.5 with sodium hydroxide. The E. coli strain ES 142 cells (ATCC 25922) were inoculated into 100 to 400 ml of LB medium and grown at 37°C with continuous shaking until the E. coli cells reached mid to late log phase of growth. The E. coli cells were collected by centrifugation at 5000 revolutions per minute (rpm) in a

GSA rotor at room temperature to form a cell pellet. The supernatant was removed from the cell pellet and the cell pellet resuspended in LB medium at a concentration of approximately $9x10^9$ bacteria per ml to form a concentrated bacteria solution. Two separate 1 ml aliquots of this solution were centrifuged in a microfuge for three minutes to form two separate bacteria cell pellets. The supernatant was separated from the cell pellets and the cell pellets were resuspended in 2 ml of the Oligomer solution prepared above and the resulting solutions immediately centrifuged to produce cell pellet and a cleared supernatant. This cleared supernatant was isolated and used as the bacteria-preadsorbed Oligomer conjugate solution. Three ml of the concentrated bacterial solution prepared above was added to this preadsorbed Oligomer conjugate solution (2 ml) to form a bacteria Oligomer uptake reaction mixture.

Table 2

Oligomers

Sequence	Patent Sequence Number
5'-GCACGCCACATC-3'	SEQ. I.D. NO.: 1
5'-A(DSSC2)*ACACGCCACATC-3'	SEQ. I.D. NO.: 2
5'-T(DSSC2)*CCTTAGCTCCTG-3'	SEQ. I.D. NO.: 3

* DSSC2 indicates the spacer described in Example 1 is positioned between those nucleotides.

(b) Preparation of Mollicutes for the Oligomer Conjugate Uptake Assay

The mollicute Acholeplasma laidlawii (ATCC 23206) was grown to stationary phase in a ATCC Media No. 243 consisting of 70% heart infusion broth (Difco, Detroit, Michigan), 20% heat-inactivated horse serum (Gibco, Grand Island, New York) and 10% yeast extract (Gibco). The mollicute cells were harvested by centrifugation, washed once with the ATCC Media No. 243. The mollicute cells were then resuspended in growth media at a final cell

concentration of approximately 5 x 10^9 cells per ml to form a concentrated mollicute cell solution.

The Oligomer conjugate prepared in Example 1 was resuspended in 2 ml of ATCC Media No. 243. The resulting solution was then preadsorbed by mixing with mollicute cells and immediately separating the mollicute cells from the solution by centrifugation. The resulting supernatant was isolated and used as the mollicute-preadsorbed Oligomer conjugate solution. 3 ml of the concentrated mollicute cell solution was added to the mollicute-preadsorbed solution to form a mollicute Oligomer conjugate uptake reaction mixture.

(c) Preparation of Eukaryotic Cells for the Oligomer Conjugate Uptake Assay

The Oligomer conjugate prepared in Example 1 was resuspended in 400 µl of RPMI 1640 media (Media Tech, Washington, D.C.) containing heat inactivated 10% fetal calf serum (Irvine Scientific, Santa Ana, California) to form an Oligomer conjugate solution. The solution was maintained at 30°C and under 5% CO_2-air atmosphere overnight to prepare it for use.

K562 cells (ATCC No. CCL240) were grown in RPMI 1640 media supplemented with 10% heat-inactivated fetal calf serum. The cells were harvested by centrifugation and then resuspended in 40 ml of the RPMI media supplemented with 10% heat-inactivated fetal calf serum so that the final cell concentration was 3.4 x 10^5 cells per ml in this cell solution.

The Oligomer conjugate was preadsorbed by mixing with K562 cells and then isolating the supernatant containing the unbound Oligomer conjugate. This supernatant was termed the preadsorbed Oligomer conjugate solution. 400 µl of this preadsorbed Oligomer conjugate solution was added to the cell solution prepared above. The resulting uptake reaction solution was maintained at 37°C under a

5% CO_2-air atmosphere during the course of the uptake assay.

(d) Oligomer Conjugate Uptake Assay

The amount of Oligomer conjugate that entered the bacterial cells, mollicute cells and eukaryotic cells was determined using an Oligomer conjugate uptake assay. This assay was performed by removing small samples from the three different uptake reaction mixtures prepared in Examples 2(a), 2(b) and 2(c) above. Three 250 or 500 μl sample aliquots were removed at various time points from the bacterial cell uptake reaction mixture and processed in the following manner. Each aliquot was centrifuged at 12,000 x g for 2 minutes to produce a bacterial cell pellet. The supernatant was separated from the cell pellet and the cell pellet was resuspended in phosphate-buffered saline (PBS) consisting of 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \bullet 7H_2O$ and 1.4 mM KH_2PO_4. 500 μl of silicon oil mix (5DC556:6DC550, William F. Nye, New Bedford, Massachusetts) was added to the resuspended cell pellet to form an admixture. This admixture was centrifuged at 12,000 x g for two minutes to form a cell pellet, an oil layer and an aqueous layer. The oil and aqueous layers were discarded and the cell pellet was resuspended in 500 μl of PBS. The resulting cell suspension was centrifuged at 12,000 x g for two minutes and the resulting cell pellet separated from the supernatant. The cell pellet was then resuspended in 500 μl 0.1 N sodium hydroxide. The resulting solution was admixed with 15 ml of Scintiverse BP fluor (Fisher Scientific, Pittsburgh, Pennsylvania). The solution was then placed in a scintillation counter and the amount of radioactivity that entered the cells detected.

Samples were also removed from the mollicute uptake reaction mixture prepared in Example 2(b) at various time points. The samples were processed according to the procedure described above for the bacterial cells except

the silicon oil mix was not used and in the wash steps, the PBS was replaced by growth media consisting of 70% heart infusion broth, 20% heat-inactivated horse serum and 10% yeast extract.

Samples were removed from the eukaryotic uptake reaction mixture prepared in Example 2(c) at various time points. The procedure used to determine the amount of Oligomer conjugate in the eukaryotic cells was as follows: Ten ml samples were removed from the eukaryotic uptake reaction mixture at various time points. The samples were centrifuged at 500 x g for 10 min. to produce a cell pellet. The supernatant was discarded and the cell pellet resuspended in 7 ml of RPMI 1640 media supplemented with 10% heat inactivated fetal calf serum. The resulting suspension was centrifuged at 500 x g for 10 min. to pellet the cells. The supernatant was discarded and the cell pellet was resuspended in 500 μl of RPMI 1640 and the resulting cell suspension layered on 500 μl of silicon oil mix in a microfuge tube. The microfuge tube was centrifuged for 2 min. and then stored at -20°C until the tube's aqueous contents were frozen. The tube was then cut below the oil layer and the portion of the tube containing the frozen aqueous layer inverted to drain away any residual fluid away from the cell pellet. The cell pellet was resuspended in 100 μl of PBS and the cells lysed by adding 100 μl of a solution containing 50% ACN and 0.2% Nonidet P-40 (NP40) (Sigma, St. Louis, Missouri). The solution was admixed with 15 ml of Scintiverse BP fluor. The solution was then placed in a scintillation counter and the amount of radioactivity that was present in the cells was determined.

3. Oligomer Conjugates Show Enhanced Entry in Bacterial Cells

The results of the bacterial cell uptake assay are shown in Tables 3A, 3B and 3C. The Oligomer conjugate 1696-1 (SEQ. ID NO. 2 conjugated with spermidine) consists of an Oligomer conjugated to spermidine via a linker. This Oligomer conjugate enters the bacterial cells at a much higher rate (Table 3B) than the unconjugated Oligomer (SEQ. ID NO. 1) (Table 3A). Another Oligomer conjugate, where the Oligomer is attached to spermine via a spacer, 1753-2 (Seq. ID No. 2 conjugated with spermine), also had an enhanced entry rate into bacterial cells (Table 3C).

Tables 3-2A and B show results of bacterial cell uptake assays for unconjugated Oligomer having a spacer (Table 3-2A) and Oligomer conjugate (Table 3-2B).

The Oligomer conjugate did not show enhanced entry into mollicute cells (Table 4) or eukaryotic cells (Table 5), relative to an unconjugated Oligomer.

Table 3

Oligomer Conjugate Uptake Into Bacteria Cells

ES142 (E. coli)

A. Unconjugated Oligomer (SEQ. I.D. NO.: 1)

time	cpm	% equilibration	μM
0	260		
6 hr.	386	19	0.11

B. Oligomer Conjugate (SEQ. I.D. NO.: 2 conjugated to spermidine)

time	cpm	% equilibration	μM
0	1799		
8 hr.	3593	95	1.5

C. Oligomer Conjugate (SEQ. I.D. NO.: 2 conjugated to spermine)

time	cpm	% equilibration	μM
0	3192		
2 hr.	4394	46	0.93
6 hr.	5601	92	1.8
22 hr.	5926	105	2.1

Table 3-2
Oligomer Conjugate Uptake Into
E. coli ES142 Cells

A. Unconjugated Oligomer incorporating C2 Spacer (SEQ. I.D. No.: 2)

time	cpm	% equilibration	μM
0	383	_	-
2 hr.	345	0	0
4 hr.	348	0	0

B. Oligomer Conjugate (SEQ. I.D. NO.: 2 conjugated to spermidine)

time	cpm	% equilibration	μM
0	813	-	-
2.5 hr.	1367	17.9	0.17
6 hr.	1546	22.0	0.20
8 hr.	1752	27.6	0.25

Table 4

Oligomer Conjugate Uptake Into

Acholeplasma laidlawii (Mollicute) Cells

Unconjugated Oligomer (SEQ. I.D. NO.: 1)

time	cpm	% equilibration	μM
0	764		
6 hr.	708	-	-
22 hr.	1730	170	1.2

Oligomer Conjugate (SEQ. I.D. NO.: 3 conjugated to spermidine)

time	cpm	% equilibration	μM
0	533		
2 hr.	583	7.2	.05
6 hr.	1018	17.5	.12
22 hr.	2334	50.7	.35

Table 5

Oligomer Conjugate Uptake Into Eukaryotic Cells

Unconjugated Oligomer (SEQ. I.D. NO.: 1)

time	% equilibration
0	6.7
1 day	26.6
2 days	32.4
3 days	39.3

Oligomer Conjugate (SEQ. I.D. NO.: 2 conjugated to spermidine)

time	% equilibration
0	5.3
1 day	47.9
2 days	46.1

4. Preparation of an Oligomer Conjugate Containing Polymyxin B

A 1 mg (30 OD Units) sample of the methylphosphonate Oligomer modified as described in Example 1(v) was dissolved in 225 μL of dimethylsulfoxide (DMSO) and 25 μL of 1 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma), pH 8, and placed in a 1 mL screw cap glass vial. The Oligomer solution was chilled to 0°C in an ice bath. Then, 10 mg of disuccinimidyl suberate (DSS) (Pierce) dissolved in 250 μL of DMSO was added to the dissolved Oligomer. The reaction was mixed for two hours at room temperature using a rotary mixer.

The reaction was then desalted by passing the solution through a 1 x 10 cm column of Sephadex L-20 (Pharmacia) that was previously swelled in 1/1 acetonitrile/water. The product was eluted with 1/1

acetonitrile/water. A single 1 mL fraction was found to contain most of the product.

To that fraction 50 μL of the 1 M HEPES buffer was added followed by 10 mg of polymyxin B sulfate (Sigma). The vessel was vortexed and sonicated to dissolve the polymyxin B. After mixing overnight, the Oligomer conjugate was desalted as described above, yielding 15 OD Units of crude product mixture in 2 mL of 1/1 acetonitrile/water.

A 30 μL aliquot was analyzed by reverse-phase HPLC on a Whatman RAC II C-18 column. The buffers used were (A) 0.15% trifluoroacetic acid (TFA) in water and (B) 0.15% TFA in acetonitrile. A gradient of 0-50% B over 50 minutes was used at a flow rate of 1 mL per minute. The chromatogram was observed at 260 nm. The product eluted at 33 minutes and comprised 74% of the product mixture as determined by integration of the chromatogram. The starting amino-labeled oligo accounted for the remaining 26% of the mixture and eluted at 25.2 minutes. The overall yield was 11 OD Units (37%). A sample was isolated using this HPLC technique. It was analyzed by mass spectrophotometry and found to be pure.

5. The Oligomer Conjugate Containing Polymyxin B Exhibits Increased Entry Into Bacterial Cells

The Oligomer conjugate containing Polymyxin B prepared above was assayed to determine its rate of entry into E. coli cells according to the methods described in Example 2. The results of this assay are shown in Table 6. These results indicate that the Oligomer conjugate containing polymyxin B has an enhanced rate of entry into E. coli cells.

Table 6

Oligomer Conjugate Containing a Membrane Disruption Molecule Entry into E. coli ES142 Cells (Seq. I.D. No. 3) Coupled to Polymyxin B

time	cpm	% equilibration	μM
0	3053	-	-
0.25 hr.	5308	429	2.02
0.5 hr.	5400	446	2.10
1 hr.	6898	731	3.44
2 hr.	8131	965	4.54

Claims

1. An Oligomer conjugate comprising:

(a) an Oligomer having a length from about 5 to about 50 nucleoside base units;

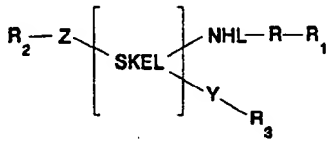
(b) an Oligomer uptake enhancer molecule; and

(c) a spacer attached to said Oligomer and to said polyamine.

2. The Oligomer conjugate of claim 1 wherein said spacer is comprised of 2 to 30 carbon atoms.

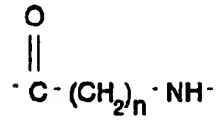
3. The Oligomer conjugate of claim 1 wherein the spacer is attached to the Oligomer at a position selected from the group: the base residue of a nucleoside, the sugar residue of a nucleoside, the 5' most nucleoside, the 3' most nucleoside, inserted between two nucleosides, and a phosphate residue.

4. The oligomer conjugate of claim 1 having the formula:



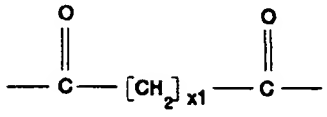
wherein SKEL comprises a non-nucleotide skeleton of about 1 to about 20 carbon atoms, wherein -NHL, Y, and Z are covalently linked to a carbon atom of SKEL; L is hydrocarbyl or a bond; Y is $-CH_2-$, -O-, -S- or -NH-; and Z is -O-, -S- or -NH-; R is hydrocarbyl or a bond; R_1 is an Oligomer uptake enhancer molecule; R_2 and R_3 are independently selected from the group consisting of a nucleoside, a nucleoside of an Oligomer, hydrogen, and a blocking group.

5. The oligomer conjugate of claim 4 wherein L comprises



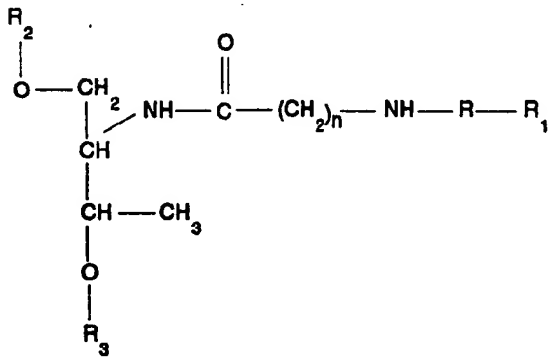
wherein n is an integer from about 1 to about 15.

6. The oligomer conjugate of claim 4 wherein R comprises



wherein X_1 is an integer from about 1 to about 15.

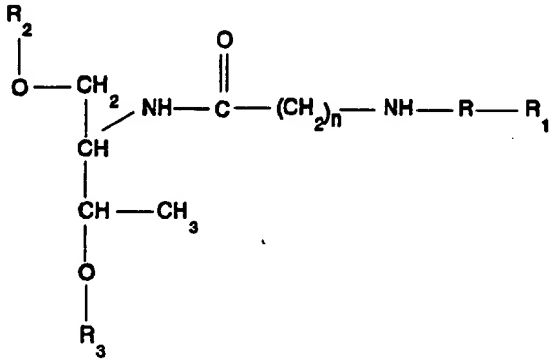
7. The Oligomer conjugate of claim 1 having the formula:



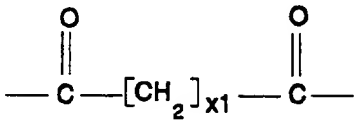
wherein n is an integer from 1 to 6; R is hydrocarbyl comprised of 1 to 20 carbon atoms; R_1 is an Oligomer uptake enhancer molecule; and R_2 and R_3 are independently selected

from the group consisting of a nucleoside, a nucleoside of an Oligomer, hydrogen and a blocking group.

8. The Oligomer conjugate of claim 1 having the formula:



wherein n is an integer from 1 to 6; and R is



wherein X_1 is an integer from 1 to 12; R_1 is an Oligomer uptake enhancer molecule; and R_2 and R_3 are independently selected from the group consisting of a nucleoside, a nucleoside of an Oligomer, hydrogen and a blocking group.

9. The Oligomer conjugate of claim 1 wherein said Oligomer is a substantially neutral Oligomer.

10. The Oligomer conjugate of claim 1 wherein said Oligomer is comprised of at least one modified nucleoside analog.

11. The Oligomer conjugate of claim 1 wherein said Oligomer comprises at least one methylphosphonate group.

12. The Oligomer conjugate of claim 1 wherein said Oligomer is a methylphosphonate Oligomer.

13. The Oligomer conjugate of claim 1 wherein said polyamine is selected from the group consisting of spermidine and spermine.

14. The Oligomer conjugate of claim 1 wherein said polyamine has the structure:

$$HR_aN[(CH_2)_nNH]_m-$$

wherein R_a is alkyl of 1 to 10 carbon atoms, n is an independently selected integer between 2 and 10 and m is an independently selected integer between 1 and 10.

15. The Oligomer conjugate of claim 1 wherein said polyamine has the structure:

$$H_2N(CH_2)_{n1}NH(CH_2)_{n2}NH(CH_2)_{n3}NH$$

wherein n_1, n_2 and n_3 are independent integers between 2 and 10.

16. The Oligomer conjugate of claim 15 wherein n_1 is 3, n_2 is 4, and n_3 is 3.

17. The Oligomer conjugate of claim 1 wherein said polyamine is of the formula:

$$H_2N(CH_2)_{n1}NH(CH_2)_{n2}\text{-}NH\text{-}$$

wherein n_1 and n_2 are independently selected integers between 2 and 5.

18. The Oligomer conjugate of claim 17 wherein n_1 is 4 and n_2 is 3.

19. A composition comprising a pharmaceutically acceptable and therapeutically effective amount of a substantially pure Oligomer conjugate according to claim 1.

20. The composition of claim 19 wherein said therapeutically effective amount is at least 0.01 percent

by weight Oligomer conjugate per weight of total composition.

21. The composition of claim 19 wherein said Oligomer conjugate is dispersed in a pharmaceutically acceptable excipient.

22. The composition of claim 19 wherein said Oligomer conjugate is dispersed in a sterile solution.

23. A method for treating an organism for an infection by a bacteria, which method comprises administering a therapeutically or prophylactically effective amount of a composition which comprises an Oligomer conjugate.

24. The method of claim 23 wherein said Oligomer conjugate comprises an Oligomer having a length from about 6 to about 50 nucleoside base units.

25. The method of claim 23 wherein said Oligomer conjugate comprises a polyamine.

26. A method of claim 23 wherein said Oligomer conjugate comprises a membrane disruption molecule.

27. The method of claim 23 wherein said organism is a mammal.

28. The method of claim 25 wherein said Oligomer and said polyamine are covalently linked together via a spacer.

29. The method of claim 26 wherein said Oligomer and said membrane disruption molecule are linked together via a spacer.

30. A method of treating an organism for a bacterial infection, which method comprises:

(a) administering to an organism an Oligomer conjugate of claim 1 comprising an Oligomer having a length from about 6 to about 50 nucleoside base units in an amount sufficient to establish within said organism a therapeutic concentration of said Oligomer conjugate; and

(b) maintaining said therapeutic amount of Oligomer conjugate within said organism for a predetermined time period sufficient to treat said organism.

31. The method of claim 30 wherein said Oligomer conjugate comprises a polyamine.

32. The method of claim 30 wherein said Oligomer conjugate comprises a membrane disruption molecule.

33. A method of preventing a bacterial infection in an organism, which method comprises:

(a) administering to an organism an Oligomer conjugate of claim 1 comprising an Oligomer having a length from about 6 to about 50 nucleoside base units in an amount sufficient to establish within said organism a prophylactic concentration of said Oligomer conjugate; and

(b) maintaining said prophylactic concentration of Oligomer conjugate within said organism for a predetermined time period sufficient to prevent a bacterial infection in said organism.

34. The method of claim 33 wherein said Oligomer conjugate comprises a polyamine.

35. The method of claim 33 wherein said Oligomer conjugate comprises a membrane disruption molecule.

36. A method of introducing an Oligomer conjugate into a bacterial cell, which method comprises:

(a) contacting an Oligomer conjugate of claim 1 comprising an Oligomer having a length from about 6 to about 50 nucleoside base units with a bacterial cell under intracellular accumulation conditions; and

(b) maintaining said contact between said Oligomer conjugate and said bacterial cell under intracellular accumulation conditions for a predetermined time sufficient for said Oligomer conjugate to enter said bacterial cell.

37. The method of claim 36 wherein said Oligomer conjugate comprises a polyamine.

38. The method of claim 36 wherein said Oligomer conjugate comprises a membrane disruption molecule.

39. A method of disinfecting an implantable medical device, which method comprises:

(a) contacting an Oligomer conjugate of claim 1 comprising an Oligomer having a length from about 6 to about 50 nucleoside base units with said implantable medical device; and

(b) maintaining said contact between said Oligomer conjugate and said implantable medical device for a predetermined time period sufficient to disinfect said implantable medical device.

40. The method of claim 39 wherein said Oligomer conjugate comprises a polyamine.

41. The method of claim 39 wherein said Oligomer conjugate comprises a membrane disruption molecule.

42. A therapeutic agent for inhibiting the growth of a target bacteria, comprising an Oligomer conjugate of claim 1 having an Oligomer of 6 to 50 nucleoside base units wherein at least a portion of the Oligomer has a

nucleic acid sequence that is substantially complementary to a portion of nucleic acid in said target bacteria.

43. A therapeutic agent for inhibiting the growth of a target bacteria, comprising an Oligomer conjugate of claim 1 containing an Oligomer of 6 to 50 nucleoside base units wherein at least a portion of the Oligomer has a nucleic acid sequence that is substantially the same as the nucleic acid sequence of the target nucleic acid sequence of a bacterial nucleic acid binding protein important for bacterial growth.

44. A therapeutic agent for inhibiting the binding of a bacterial protein to its target nucleic acid comprising an Oligomer conjugate of claim 1 containing an Oligomer of 6 to 50 nucleoside base units wherein at least a portion of the Oligomer has a nucleic acid sequence that is substantially the same as a portion of the nucleic acid sequence of the target nucleic acid.

45. A therapeutic agent for selectively binding a nucleic acid inside a bacteria comprising an Oligomer conjugate of claim 1 containing an Oligomer of 6 to 50 nucleoside base units wherein at least a portion of the Oligomer has a nucleic acid sequence that is substantially complementary to at least a portion of said nucleic acid.

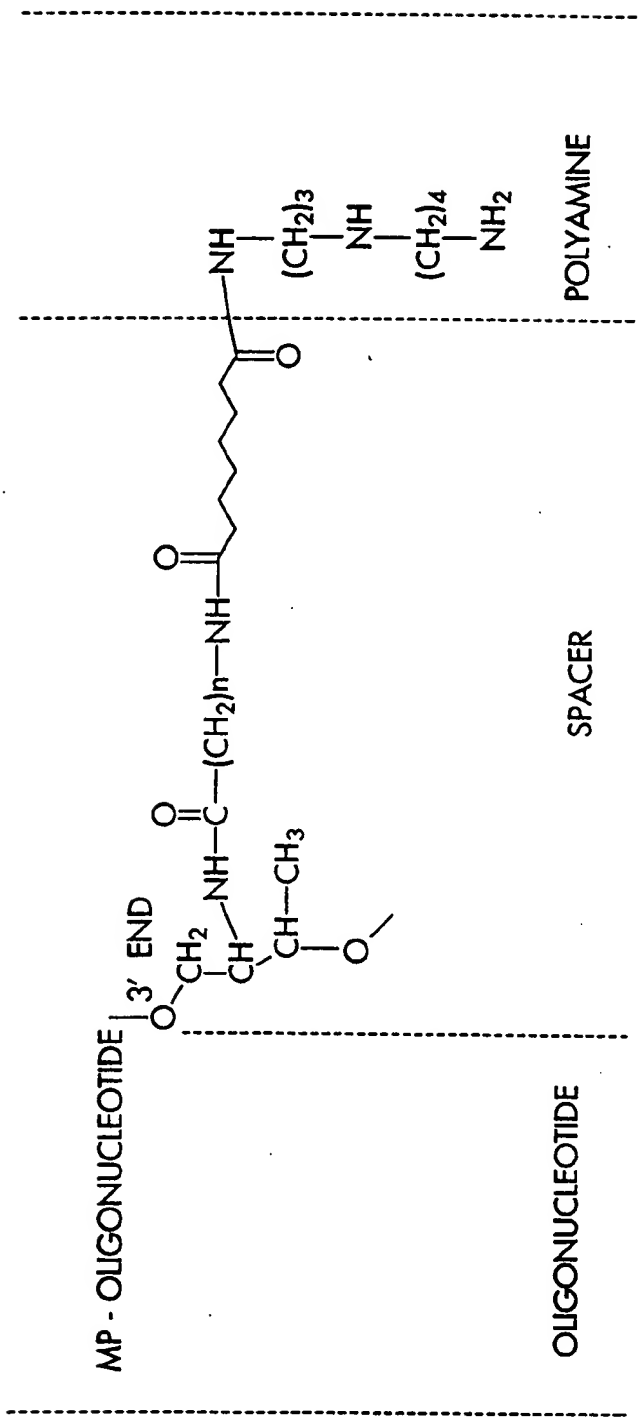

MP - OLIGONUCLEOTIDE
3' END
OLIGONUCLEOTIDE
SPACER
POLYAMINE

FIG. 1

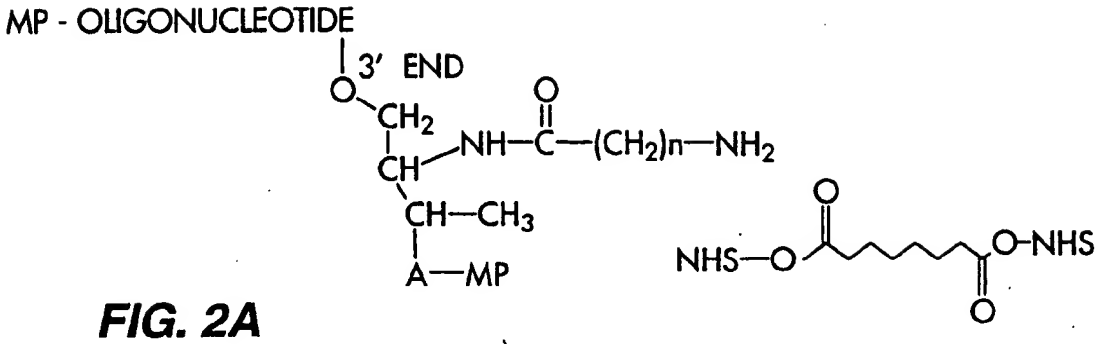


FIG. 2A

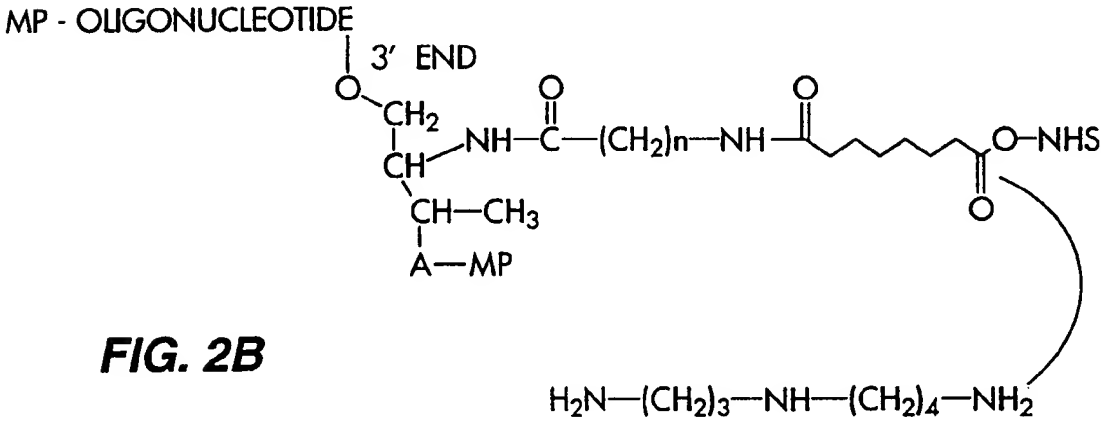


FIG. 2B

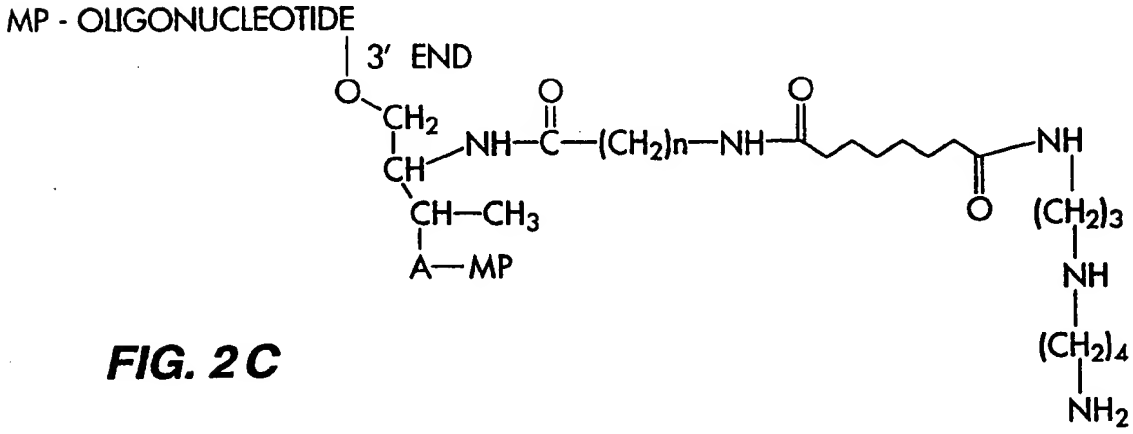


FIG. 2C

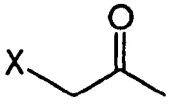
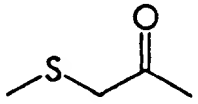
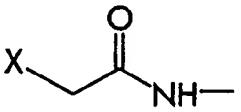
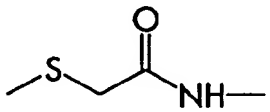
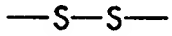
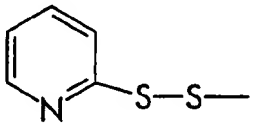
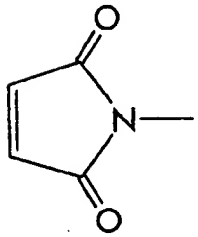
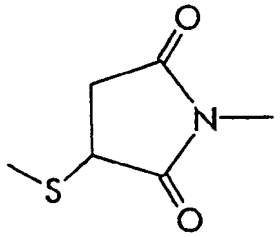
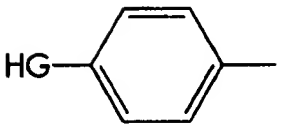
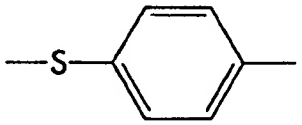
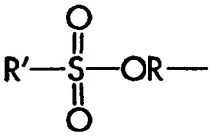
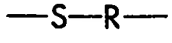
Y	Z		YZ
— SH		HALOACETYL X = I, Br	
		HALOACETAMIDE X = I, Br	
		DISULFIDES *i.e.*	
			
		MALEIMIDES	
		ARYLMERCURY COMPOUNDS	
		SULFONYL	

FIG. 3a

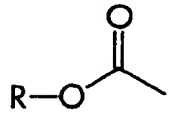
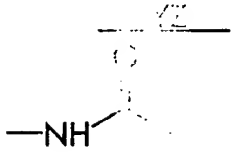
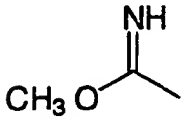
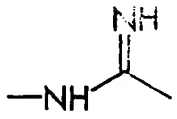
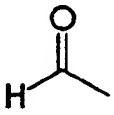
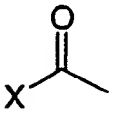
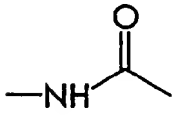
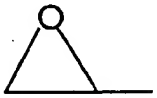
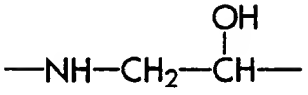
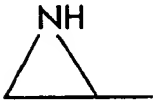
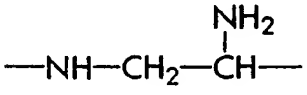
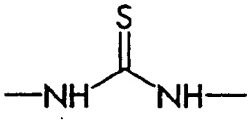
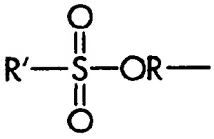
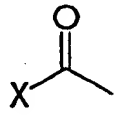
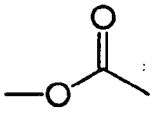
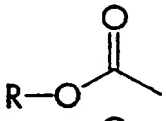
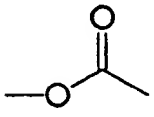
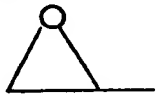
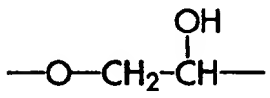
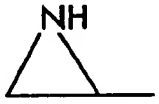
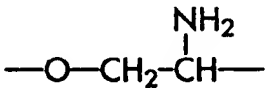
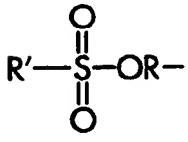
Y	Z		
$-NH_2$		ACTIVE ESTERS	
		METHOXY IMINES	
		ALDEHYDES (TO FORM SCHIFF BASES FOLLOWED BY REDUCTION)	$-NH_2-CH_2-$
		ACID HALIDES X=HALOGEN	
		EPOXIDES	
		AZIRIDINES	
	SCN—	THIOCYANATES	
		SULFONYL	—NH—R—
—OH		ACID HALIDES X=HALOGEN	
		ACTIVE ESTERS	
		EPOXIDES	
		AZIRIDINES	
		SULFONYL	—O—R—

FIG. 3b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05568

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 31/70, 31/80, 37/02; C07H 23/00
US CL :422/1; 424/93D; 514/44; 536/25.3, 25.6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/1; 424/93D; 514/44; 536/25.3, 25.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, A, 5,138,045 (COOK ET AL) 11 August 1992, see entire document.	1-3, 15 ---------- 4-8, 9-14, 16-45
Y	WO, A, 92/02532 (REYNOLDS ET AL) 20 February 1992, see entire document.	4-8, 19-45
Y	Microbiological Reviews, Volume 49, Number 1, issued March 1985, Tabor et al, "Polyamines in Microorganisms", pages 81-99, see pages 82-88, 92, 93.	9-12, 14, 16-18, 19-45
Y	Nucleic Acids Research, Volume 11, Number 18, issued September 1983, Miller et al, "Preparation of Oligodeoxyribonucleotide Methylphosphonates on a Polystyrene Support", pages 6225-6242.	9-12, 14, 16-18, 19-45

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search
22 JULY 1994

Date of mailing of the international search report
SEP 08 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05568

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Derwent WPI, WPI Acc No: 90-051727/07, WO, A, 90/00624, (LUPSKI) 25 January 1990, see entire abstract.	13, 19-45
Y	Bioorganicheskaia Khimiia, Volume 16, Number 4, issued April 1990, Gottikh et al, "Design of New Oligonucleotide Derivatives Resistant to Cell Nucleases Degradation", pages 514 and 523, see entire abstract, page 523.	1-45
A	Microbiological Reviews, Volume 56, Number 3, issued September 1992, Vaara, "Agents that Increase the Permeability of the Outer Membrane", pages 395-411, see entire document.	1-45